THE EFFECT OF BREWED TEA ON NONHEME IRON AVAILABILITY FROM RICE-BASED TEST MEALS BY *IN VITRO* METHOD

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NUTHATHAI SUTTHIWONG : THE EFFECT OF BREWED TEA ON NONHEME IRON AVAILABILITY FROM RICE-BASED TEST MEALS BY *IN VITRO* METHOD. THESIS ADVISORS : NAIYANA BOONTAVEEYUWAT, Ph.D.(Biotecnology), SOMCHAI DURONGDAJ, Ed.D. 105 p. ISBN 974-04-1228-9

The bioavailability of dietary nonheme iron is influenced by enhancers and inhibitors of iron absorption in the diet. Some of the major inhibitors of iron absorption are the phenolic compounds, particularly tannic acid, which is commonly found in tea. This study was conducted by adding various volumes of brewed tea, 100 ml, 200 ml, and 300 ml, in polished, mixed, and brown rice-based test meals to investigate the inhibitory effect of brewed tea on the availability of dietary nonheme iron using an *in vitro* method.

Adding 100, 200, and 300 ml of brewed tea reduced iron availability from the polished rice-based test meal by 27%, 14% and 4%, the mixed rice-based test meal by 18%, 10% and 4% and the brown rice-based test meal by 17%, 9% and 6%. The results showed that the inhibition of iron availability by brewed tea was not volume-related. It was probably due to the brewed tea having a high availability of iron (24.98%), so that the greater volume of brewed tea added, the greater the available iron presented in the test meal. However, there were no significant differences between the effects of 100, 200, and 300 ml of brewed tea ($p>0.05$). Two major aspects that might have affected the inhibitory effect of brewed tea were the amount of tannic acid and vitamin C in the test meal. Firstly, the test meal had a high amount of tannic acid, approximately 83.5 mg per meal, corresponding to a 20:1 ratio of tannic acid to iron. The iron might have combined with tannic acid until available iron hardly remained, so that increasing tannic acid in a test meal, by adding brewed tea, did not further decrease iron availability. Secondly, the test meal had a high amount of vitamin C, approximately 133.5 mg per meal, which is a strong enhancer of iron absorption. Not only affecting the inhibitory effect of brewed tea, vitamin C might interfere with the effect of phytate on iron availability as it could cause the three types of rice-based test meal to have no significant difference in iron availability ($p>0.05$).

The present study suggests that brewed tea consumption might have little effect on iron absorption in persons who consume meals containing a high ratio of tannic acid to iron and a high amount of vitamin C. However, vulnerable groups, such as children, pregnant and lactating women, should not consume brewed tea with meals. In addition, people who favor consuming brown rice, which has high phytate, should avoid consuming brewed tea with meals to reduce the risk of obtaining more iron absorption inhibitors.

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ณัฐหทัย สุทธิวงษ์ : ผลของน้ำชาต่อปริมาณธาตุเหล็กที่ใช้ประโยชน์ได้จากอาหารที่มีข้าวเป็นส่วนประกอบหลัก โดยการศึกษาในหลอดทดลอง (THE EFFECT OF BREWED TEA ON NONHEME IRON AVAILABILITY FROM RICE-BASED TEST MEALS BY *IN VITRO* METHOD) คณะกรรมการควบคุมวิทยานิพนธ์: นัยนา บุญทวีวุฒน์, วท.ด.(เทคโนโลยีชีวภาพ), สมชาย ดุรงค์เดช, Ed.D. 105 หน้า. ISBN 974-04-1228-9.

การนำธาตุเหล็กที่ไม่ใช่ฮีมจากอาหารไปใช้ประโยชน์ในร่างกายได้รับอิทธิพลจากตัวส่งเสริมและตัวยับยั้งการดูดซึมธาตุเหล็กที่มีอยู่ในอาหารนั้นๆ ด้วย โดยตัวยับยั้งการดูดซึมธาตุเหล็กที่สำคัญชนิดหนึ่งคือ สารประกอบโพลีฟีนอล โดยเฉพาะอย่างยิ่งกรดแทนนิคซึ่งมีในชา การวิจัยครั้งนี้ได้เติมน้ำชาปริมาตรต่างๆ ลงในอาหารทดลองที่มีข้าวเป็นส่วนประกอบหลัก 3 ชนิดเพื่อศึกษาผลของน้ำชาต่อการนำธาตุเหล็กที่ไม่ใช่ฮีมไปใช้ประโยชน์โดยการใช้วิธีในหลอดทดลอง

การเติมน้ำชา 100, 200 และ 300 มล. ลดปริมาณธาตุเหล็กที่ใช้ประโยชน์จากอาหารที่มีข้าวเจ้าขัดสีเป็นส่วนประกอบหลักร้อยละ 27, 14 และ 4 จากอาหารทดลองที่มีข้าวผสมเป็นส่วนประกอบหลักร้อยละ 18, 10 และ 4 และจากอาหารที่มีข้าวกล้องเป็นส่วนประกอบหลักร้อยละ 17, 9 และ 6 ตามลำดับ จากผลการทดลองพบว่าผลการยับยั้งของน้ำชาต่อการนำธาตุเหล็กไปใช้ประโยชน์ไม่สัมพันธ์กับปริมาตรน้ำชา อาจเนื่องมาจากน้ำชาที่ใช้ในการทดลองนี้มีธาตุเหล็กที่ใช้ประโยชน์ได้สูง (ร้อยละ 24.98 ของธาตุเหล็กรวม) ดังนั้นเมื่อเติมน้ำชาลงในอาหารเพิ่มขึ้นปริมาณธาตุเหล็กที่ใช้ประโยชน์ได้จึงเพิ่มขึ้นด้วย อย่างไรก็ดีผลการยับยั้งของน้ำชาปริมาตร 100, 200 และ 300 มล. แตกต่างกันอย่างไม่มีนัยสำคัญ ($p>0.05$) ซึ่งน่าจะมาจากสาเหตุที่เป็นไปได้ 2 สาเหตุหลัก คือ ปริมาณกรดแทนนิคและวิตามินซีในอาหารทดลอง สาเหตุแรก คือ อาหารทดลองนี้มีปริมาณกรดแทนนิคสูง (83.5 มก. ต่ออาหาร 1 ชุด) ซึ่งคิดเป็นอัตราส่วนของกรดแทนนิคต่อธาตุเหล็กได้ 20:1 ธาตุเหล็กอาจจับกับกรดแทนนิคจนแทบไม่เหลือธาตุเหล็ก ดังนั้นเมื่อเพิ่มกรดแทนนิคลงในอาหารโดยการเติมน้ำชา ปริมาณธาตุเหล็กที่ใช้ประโยชน์ได้จึงไม่ลดลงเพิ่มมากขึ้น สาเหตุที่ 2 คือ อาหารทดลองนี้มีปริมาณวิตามินซีสูง (133.5 มก. ต่ออาหาร 1 ชุด) ซึ่งวิตามินซีนั้นเป็นตัวส่งเสริมการดูดซึมธาตุเหล็กที่สำคัญ นอกจากอาจจะส่งผลต่อการยับยั้งของน้ำชาแล้ววิตามินซีก็ยังอาจจะส่งผลต่อผลของไฟเตทต่อการนำธาตุเหล็กไปใช้ประโยชน์ โดยทำให้การนำธาตุเหล็กไปใช้ประโยชน์จากอาหารที่มีข้าวเป็นส่วนประกอบหลัก 3 ชนิดแตกต่างกันอย่างไม่มีนัยสำคัญทางสถิติด้วย ($p>0.05$)

จากผลของการศึกษาครั้งนี้อาจบอกถึงแนวโน้มของผลของน้ำชาต่อการดูดซึมธาตุเหล็กได้ว่า การบริโภคน้ำชาอาจมีผลต่อการดูดซึมธาตุเหล็กเพียงเล็กน้อยในคนที่บริโภคอาหารที่มีอัตราส่วนของกรดแทนนิคต่อธาตุเหล็กสูงอยู่แล้ว อย่างไรก็ตามในผู้ที่มีความเสี่ยงต่อการขาดธาตุเหล็ก เช่น เด็ก หญิงตั้งครรภ์ และหญิงให้นมบุตรไม่ควรดื่มน้ำชาร่วมกับมื้ออาหาร นอกจากนั้นในคนที่นิยมบริโภคข้าวกล้องซึ่งมีไฟเตทปริมาณมากนั้น ควรหลีกเลี่ยงการดื่มน้ำชาร่วมกับมื้ออาหารนั้น เพื่อลดโอกาสที่จะได้รับปัจจัยยับยั้งการดูดซึมธาตุเหล็กเพิ่มขึ้น

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CHAPTER I

INTRODUCTION

Background and rationale

Anemia is approximately found in 2.2 billion individuals worldwide, half of whom are caused by iron deficiency (1). In most areas of the world, including Thailand where iron deficiency anemia remains a major problem (2), iron deficiency is prevalent among primary infants and young children because of a higher iron requirement related to growth, and women of childbearing age as a result of menstrual loss and pregnancy. Iron deficiency decreases immune function, diminished work capacity, and increased risk of delivering preterm and low-birth-weight infant (3). A major cause of iron deficiency is low dietary iron bioavailability intake because most of the iron in diets is usually, 80-90 percent, present as nonheme iron. In Thailand, Boontaveeyuwat (4) reported that nonheme iron content of the urban Thai diet was approximately 93 percent, and 95 percent in the rural Thai diet.

The bioavailability of dietary nonheme iron is influenced by enhancers and inhibitors of iron absorption (5). One main inhibitor of iron absorption are the phenolic compounds, including phenolic acid, flavonoids and their polymerisation products. They are widely present in fruits, certain spices, vegetables, and particularly high in beverages such as tea. Polyphenolic compounds from all three classes have been shown to inhibit iron absorption but to different extents, with the hydrolysable tannins, tannic acid, of tea being the most potent inhibitors (6-8) because the galloyl groups

in tannic acid molecule can directly form complexes with iron in the gastrointestinal lumen. Brune *et al.* (7) found the foods and beverages containing galloyl group inhibited iron absorption approximately in proportion to the respective content of galloyl groups, expressed as tannic acid equivalents. Because of the high tannic acid equivalent, tea is the most potent inhibitor of iron absorption.

Brewed tea consumption spread worldwide to countries such as China, Japan, India and Western countries, and the tea plant is currently cultivated in approximately 30 countries. About three billion kilograms of tea are produced and consumed yearly. Consumption levels of brewed tea vary widely around the world; it is believed that tea consumption is the second only to water, with consumption of approximately 120 ml per person per day (9-11). Although the consumption of brewed tea in Thailand is not common, some groups of people like to consumed brewed tea i.e. Chinese who live in Thailand, older people and monks. Other groups of people who get brewed tea are people who dine in restaurant where brewed tea is served, such as Suki Yaki restaurants and noodle shops. Some vegetables are rich in tannic acid. Thai diets are high vegetable diets, so they have some amount of tannic acid. People who usually drink brewed tea after meal, they would get more tannic acid.

Another main inhibitor of iron absorption is the phytate present in cereals, vegetables, roots, nuts, etc. Cook *et al.* (12) studied the influence of cereal grains on iron absorption from infant cereal foods. They found there was a strong inverse correlation between iron absorption and the phytate content of different cereals. Thus, people who usually consume cereals, vegetables or some diets that have phytate and tea together would intake more inhibitors of nonheme iron absorption.

Rice is a common composition of Thai meals that is high in phytate. At present, some Thai people regularly consume polished rice, someone consumes brown rice and someone who begin to consume brown rice but are not familiar with the taste often mix it with polished rice. The consumption of Thai meals are therefore composed commonly of three types of rice, polished, brown and mixed rice. If some beverage has nonheme iron absorption inhibitors, which were taken during a meal, what was the effect of it on nonheme iron availability from different rice-based test meal. Thus, the present study investigated the effect of brewed tea on nonheme iron availability from all three types of rice-based test meal.

General objective

To study the effect of brewed tea on nonheme iron availability from three types of rice-based test meal.

Specific objectives

1. To study the effects of brewed tea in the volume of 100, 200 and 300 ml, on nonheme iron availability from the same type of rice-based test meal.

2. To study the availability of nonheme iron from three types of rice-based test meal at the same volume of brewed tea added.

Hypothesis

1. The more volume of brewed tea added, the lower iron availability from rice-based test meal is found.

2. At the same volume of brewed tea added, iron availability from the test meal with more amount of polished rice is higher than that from the test meal with lesser amount of polished rice.

Scope of study

The study determined the effect of brewed tea on the availability of nonheme iron. Different volumes of brewed tea, 100, 200 and 300 ml, were added into a reference rice-based test meals which composed of vegetables and different types of rice. Three kinds of vegetable, string bean, kidney bean and Thai spinach, were mixed with three types of rice, polished, mixed and brown rice. The control chemical compositions on total iron (5 mg) and crude fiber (5 g) were fixed in all types of rice-based test meal. Other chemical compositions both vitamin C and tannic acid contents in all types of rice-based test meal were the same.

Definition terms

1. **Total iron** was the amount of all forms of iron, Fe^{2+} and Fe^{3+}, from test meal (mg/100 g).

2. **Available iron** was the dialysable iron under simulated gastrointestinal conditions by *in vitro* method. After enzyme digestion step, available iron was measured by atomic absorption technique.

3. **Rice-based test meal** was the meal that was composed of rice 60 g and the 3 kinds of vegetable. The vegetable contents were 150 g of yard long bean, 100 g of kidney bean and 50 g of Thai spinach. The amounts of total iron, crude fiber, vitamin C and tannic acid in all basic rice-based test meals were of the same quantity.

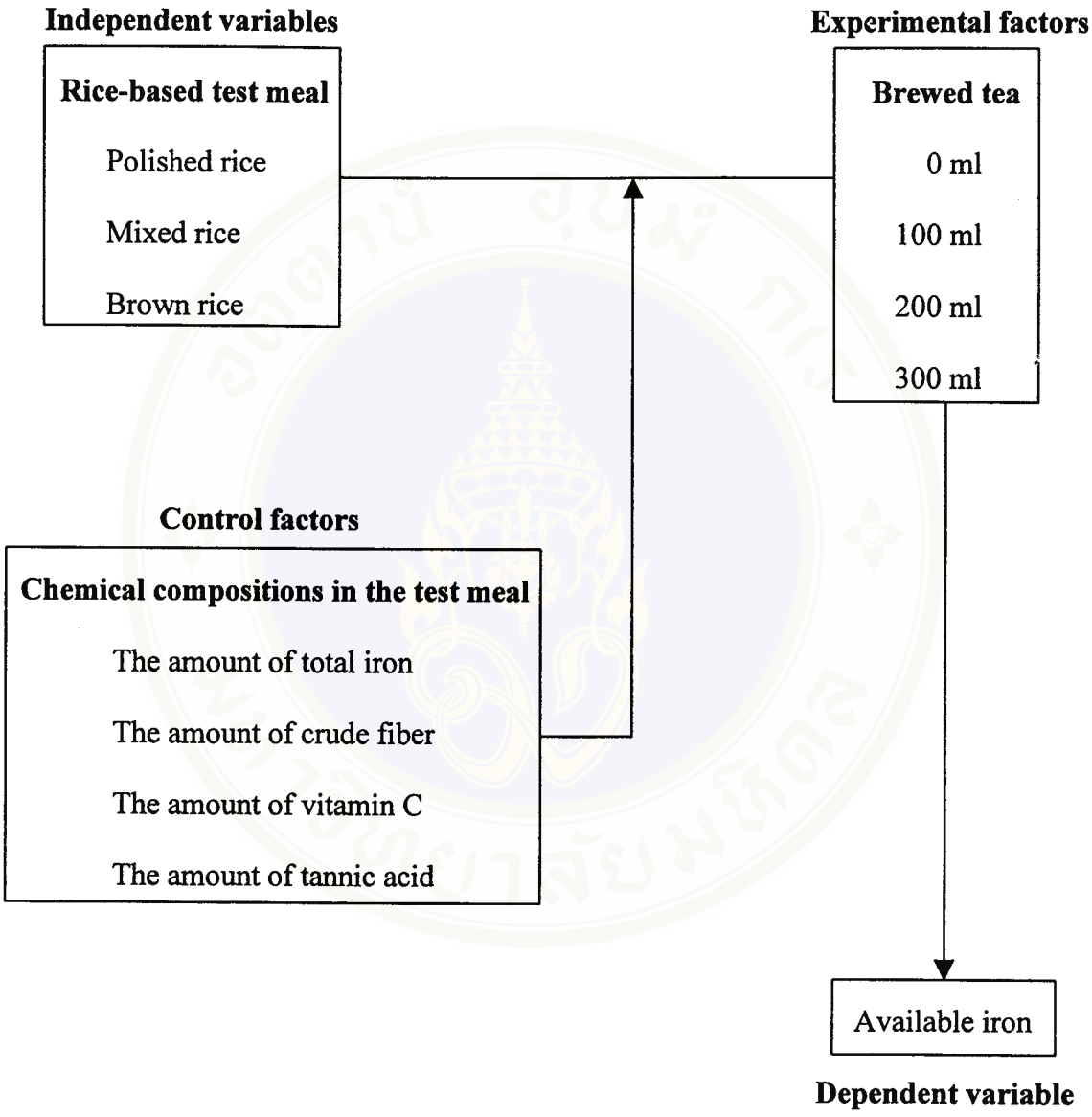
4. **Polished rice-based test meal** was the test meal that was composed of polished rice 60 g and the 3 kinds of vegetable.

5. **Mixed rice-based test meal** was the test meal that was composed of 2 kinds of rice, polished rice 30 g and brown rice 30 g, and the 3 kinds of vegetable.

6. **Brown rice-based test meal** was the meal that was composed of brown rice 60 g and the 3 kinds of vegetable.

7. **Tannic acid equivalent** was content of galloyl groups that potentially bind with iron from reagent to be Fe-galloyl group

Conceptual framework



CHAPTER II

LITERATURE REVIEW

The role of iron in the human body

Iron is a micronutrient for the body which is a component of hemoglobin, myoglobin and other hemoprotein. The body should receive iron from foods sufficient for requirements because iron appears in regular meals such as cereals and flour products which we have regularly, and also in a variety of vegetables which can easily be found in the market at a low price. However, many people can be found with iron deficiency anemia among those of low socio-economic status. The main cause of iron deficiency is nutritional, as the diet cannot meet physiological requirements.

The total amount of iron in the body is about 2,000-3,000 mg in adults. Since iron is continuously recycled in the body, the daily iron requirement is relatively small. The amount of iron would be replaced for that lost from the body and necessary for growth and development.

The requirement of dietary iron in each person is different. The iron demand is greatest during the first three years of life, which is a period of rapid growth. Females require iron more than males. The female group, including normal, pregnant and lactating women, is one of the high risk groups for iron deficiency due to the menstruation cycle, abnormal bleeding, pregnancy and the period of milk production during lactation. The iron requirements for individuals recommended by the Thailand Recommended Daily Dietary Allowances (13) are shown in Table 1.

Table 1 Thailand Recommended Daily Dietary Allowances (mg/day)

Groups	Ages (years)	Iron requirement (mg)
Infant*	< 3	Breast milk
	3-5	6
	6-8	7
	9-11	8
Child	1-3	10
	4-6	10
	7-9	10
Boy	10-12	12
	13-15	12
	16-19	10
Girl	10-12	15
	13-15	15
	16-19	15
Man	20-29	10
	30-39	10
	40-49	10
	50-59	10
	60+	10
Woman	20-29	15
	30-39	15
	40-49	15
	50-59	10
	60+	10
Pregnant		+30
Lactating		+15

* Age in months of infant

Factors controlling iron absorption

1. **Types of food iron:** Food iron exists into 2 forms, heme and nonheme iron.

1.1 Heme iron

Heme iron derived from hemoglobin and myoglobin, mainly in meat products, comprises approximately 30 to 40 percent of the iron in pork, liver and fish and 50 to 60 percent of the iron in beef, lamb and chicken (14-15). Twenty-three percent of heme iron can be absorbed (16), and the absorption is not dependent on the interaction of the composition within the diet (17).

1.2 Nonheme iron

Nonheme iron exists in both the organic compounds present in foods, and the inorganic compounds known as iron salt. Nonheme iron is derived mainly from cereals, vegetables, and fruit (17). All iron in plants is nonheme iron, while only 60 percent of total iron in animal foods is nonheme iron. The body can absorb 3 to 8 percent of nonheme iron (16). The bioavailability of nonheme iron in any diet is dependent on the interaction of the enhancers and inhibitors of iron absorption contained within the diet (18-21).

2. **Factors affecting iron absorption**

The amount of nonheme iron absorbed from the diet is determined not only by its iron content but also by the interaction of enhancing or inhibiting factors of iron absorption contained within the diet.

2.1 Enhancers of iron absorption

2.1.1 Vitamin C

Vitamin C or ascorbic acid is the best known and most potent enhancer of iron absorption both in its natural form in fruit and vegetables (22), and when added as the free compound (23). The enhancing effect is dose-related over the range 25-1000 mg (23). Ascorbic acid increased the bioavailability of all iron fortification compounds (24) but is sensitive to losses during storage and cooking. Its facilitating effect is thought to be due to its ability to convert ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) at low pH, and its chelating properties (25).

2.1.2 Meats

Many studies have confirmed the enhancing effect of meats or muscle tissue on iron absorption (19, 26, 27). Beef, lamb, chicken, fish, pork and liver also have an enhancing effect which appears to be related to the high level of cysteine-containing proteins in these tissues. Cysteine is the only free amino acid to have a similar enhancing effect on iron absorption in humans (28) and at equivalent quantities of cysteine, free cysteine, gluthathione or beef similarly increased iron absorption from a maize meal (29). The facilitating effect of an enzymatically-digested beef extract on iron absorption was removed by oxidating the cysteine residues before feeding (30). Their potential to chelate iron and to reduce ferric iron to the more soluble ferrous iron, could explain the enhancing effect of cysteine-containing peptides on iron absorption.

2.2 Inhibitors of iron absorption

2.2.1 Phytate

Phytate, phytic acid or myoinoitol hexadihydrogen phosphate, exists wholly as a soluble salt (Figure 1). This organic salt reduces the absorption of divalent and trivalent cations by combining with them to form an insoluble salt (31-32). This makes for a reduction of their availability for absorption. Phytate is widely present in cereal grain and legume seeds. Phytate has been found to be very high in pulses, nuts, dry grains and also seeds that could be bred (33). It is the major factor in the low absorption of iron from these foods. It accumulates under the bran of cereal grains and is significantly reduced through milling. The inhibitory effect of bran on iron absorption was almost completely removed by degrading phytic acid (34), whereas adding phytic acid to wheat rolls made from white flour inhibited iron absorption in a dose-dependent fashion (35).

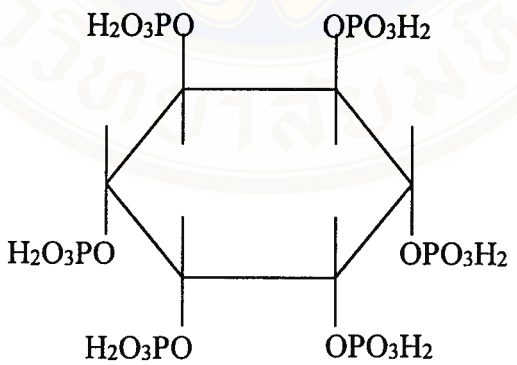


Figure 1 Chemical structure of phytate

2.2.2 Calcium

Calcium (Ca) can inhibit iron absorption when fed as an inorganic calcium compound or when consumed in dairy products such as milk or cheese. The level of inhibition depends on the quantity of calcium consumed and on the size and

composition of the meals, being more pronounced in small, simple meals such as bread rolls or hamburgers, but may not exist in larger, more complex, meals. In a small bread meal, inhibition of iron absorption was dose-related up to 300 mg calcium added as calcium chloride, and was similarly (50-60%) inhibited for 165 mg calcium added as the inorganic calcium compound or as 150 ml milk (36). However, 250 ml milk (275 mg Ca) caused only a modest, non-significant 14% fall in iron absorption from a more complex meal of hamburger, potato and string beans (37) and 150 ml milk added to a typical French meal of steak, carrots, French fries, camembert cheese, apple, bread and water had no effect (38).

2.2.3 Polyphenols

The phenolic compounds present in plant foods include phenolic acid, flavonoids and their polymerisation products. They are particularly high in beverages such as tea, coffee, herb teas, cocoa and red wine. Polyphenolic compounds from all three classes have been shown to inhibit iron absorption, but to different extents, with the tannins being the most potent inhibitors.

Tannins

Tannins are compounds of intermediate to high molecular weight. They can combine with iron for Fe-phenolic complex formation in the gastro-intestinal lumen, which makes the nonheme iron less available for absorption (7).

1. Groups of plant tannin

1.1 Hydrolyzable tannins

Hydrolyzable tannins are polymers of gallic or ellagic acid. These contain a central core of polyhydric alcohol such as glucose, and hydroxyl groups.

Hydrolyzable tannins are hydrolyzed with acid, alkali, and hot water. The best-known hydrolyzable tannin is tannic acid, which is a gallotannin consisting of a glucose molecule that can further esterify with another ten gallic acid (Figure 2) units (39).

1.2 Condensed tannins

Condensed tannins or proanthocyanidins are structurally more complex than hydrolyzable tannins (40). They are mainly the polymerized products of flavan-3-ol (catechin, epicatechin etc.) and flavan-3,4-diols, or a mixture of the two. Condensed tannins are found mainly in fruit, vegetables, grains and legumes. Condensed tannins usually accumulates in the outer layer of plants (41).

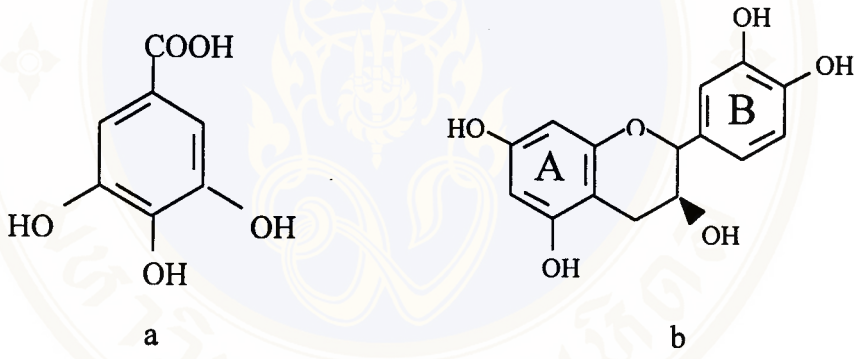


Figure 2 Chemical structures of gallic acid (a) and catechin (b)

2. Effect of tannins on available iron

2.1 Effect of synthetic tannins

Rao *et al*, 1982 (42) detemined the effect of adding tannin (tannic acid) to foods containing no tannin and to a ferric chloride solution on ionisable iron. When tannin was added to a ferric chloride solution, there was an initial increase in ionisable iron (5.98-17.95 mg tannin) when the tannin concentration was increased beyond this, gradually decreased. At the amount of tannin 47.87 mg, it could inhibit ionisable iron 50 percent. However, when tannin was added to foods which do not normally contain

any native tannin, there was no initial change in ionisable iron at low tannin concentrations (12.8 and 25.5 mg tannin). When tannin was 125.6 to 502.0mg, ionisable iron was innibited by tannic acid 33 to 67 percent.

Gillooly *et al*, 1983 (43) studied the effect of tannic acid on the absorption of iron from vegetables. This study added one quantity of tannic acid, 500 mg, to a broccoli meal. Tannic acid caused marked inhibition of iron absorption from 0.297 mg to 0.015 mg equal to 95 percent inhibition.

Brune *et al*, 1989 (7) demonstrated the relationship between iron absorption and the amount and type of phenolic compounds from wheat roll meals. In a study of the effect of small phenolic compounds with different hydroxylation patterns, gallic acid inhibited iron absorption to the same extent as tannic acid, per mol galloyl groups, whereas no inhibition was observed when catechin was added to the test meal. Chlorogenic acid inhibited iron absorption to a lesser extent. The inhibition of iron absorption by tannic acid was strongly dose-related. The smallest amount of tannic acid, 5 mg, inhibited absorption by 20 percent, 25 mg by 67 percent and 100 mg by 88 percent.

Brown *et al*, 1990 (44) studied the relative effects of tannic acid on iron absorption in rats when administered by esophageal cannula or by meal. Tannic acid reduced iron absorption from 82.0 percent to 63.9 percent by esophageal cannula and to 53 percent by meal. The study of pure polyphenols e.g. epicatechin, chlorogenic acid, gallic acid, tannic acid and caffeic acid, all the standard polyphenol compounds, except epicatechin, were inhibitory. Tannic acid (25.2% inhibition) and gallic (25.4%

inhibition) were the most inhibitory when compared to the water control, and they had an influence on iron absorption more than chlorogenic acid and caffeic acid.

Seigenberg *et al*, 1991 (45) studied the effect of synthetic hydrolyzable tannin, tannic acid, on nonheme iron absorption. The effect of increasing doses of tannic acid on iron absorption from a wheat bread meal when a small amount of tannic acid was added were lower than when a large amount of tannic acid was added. A significant inhibitory effect was noted with as little as 12mg tannic acid. The inhibitory effect was greater with 26 and 55mg tannic acid and was profound with 263 and 833mg. Absorption ratios indicated that maximal inhibitory effect was achieved between 12, 26 and 55mg tannic acid, with the ratio dropping to 0.7, 0.48 and 0.33 or 30, 52, and 67 percent inhibition, respectively. The dose-response curve then leveled off, even with very large doses (263 and 833mg) of tannic acid.

The results from these previous studies shown the effect of synthetic tannins, tannic acid, and phenolic acids on ionisable iron or iron absorption. Gallic acid, a phenolic acid with one galloyl group and tannic acid inhibited iron absorption to the same extent. Chlorogenic acid and caffeic acid could inhibit iron absorption less than gallic acid and tannin acid, whereas catechin, a flavanol made up of the catechol and resorcinol group, and epicatechin did not affect iron absorption.

2.2 Effect of food tannins

Disler *et al*, 1975 (6) demonstrated the effect of tea on iron absorption from a bread meal. When volunteer subjects consumed tea 200 ml which was prepared from 5g dry tea, it could inhibit nonheme iron absorption by 68 percent. Iron

Table 2 Summary of the effect of synthetic tannins from previous studies

Researcher	Method	Meal	Synthetic tannin	Result
Rao *et al*, 1982 (42)	*In vitro*	Wheat	Tannic acid 0-628 mg	Decrease ionisable iron
		Ferric chloride solution	Tannic acid 0-119.68 mg	Decrease ionisable iron when tannic acid 23.94 -119.68 mg
Gillooly *et al*, 1983 (43)	*In vivo*	Broccoli	Tannic acid 500 mg	Reduced 95%
Brune *et al*, 1989 (7)	*In vivo*	Wheat roll	Gallic acid 14.7 mg	Reduced 52%
			Chlorogenic acid30.5mg	Reduced 33%
			Catechin 25 mg	Not affect
			Tannic acid 5-100 mg	Reduced 20-94%
Brown *et al*, 1990 (44)	In rats	Meal	Epicatechin 20 mg	Not affect
			Chlorogenic acid 20 mg	Reduced 16%
			Gallic acid 20 mg	Reduced 25.4%
			Tannic acid 20 mg	Reduced 25.2%
			Caffeic acid 20 mg	Reduced 12.7%
Siegenberg *et al*, 1991 (45)	*In vivo*	White tannic acid-free bread	Tannic acid 12-55 mg	Reduced 34-81%

absorption from solutions of $FeCl_3$ and $FeSO_4$ were inhibited when tea was added by 29 or 71 percent.

Rossander *et al*, 1979 (46) had human volunteers consume a breakfast meal which was composed of wheat roll with margarine, orange marmalade and cheese. When subjects drank tea (2.5 g of dry tea allowed to steep in 150 ml of boiled water for 5 minutes) with a breakfast meal the absorption decreased from 0.16 to 0.07mg or

56 percent inhibition. Serving tea instead of coffee in a breakfast containing egg and bacon reduced absorption by 52 percent.

Kojima *et al*, 1981 (47) examined the solubilization of iron from cooked pinto beans by using on *in vitro* methodology. In examining the effect of beverages this study used a dual incubation procedure. Ten ml of the beverage was taken to pH 2 and mixed with 10 ml of the cooked pinto bean suspension. The mixture was incubated for 30 minutes. The mixture was taken to pH 6 and reincubated. Regarding the amount of iron released to the supernatant, tea markedly decreased the amount of iron found in the supernatant. Only 12 percent of the iron that would be spontaneously soluble remained in solution in the presence of tea. A 20 percent ethanol solution caused a slight decrease in the soluble iron. Both freshly brewed coffee and instant coffee have little effect on the total soluble iron.

Hallberg *et al*, 1982 (48) studied the effect of various drinks on the absorption of nonheme iron. The drinks were taken with standard meals composed of a hamburger, string beans and mashed potatoes. The tea was prepared by using 4 g of dry tea which was allowed to steep in 250ml of boiled water for 5 minutes. The coffee was brewed using 14g coffee to 250ml water. A reduction in iron absorption was seen serving tea (62%) or coffee (35%) with the meal.

Rao *et al*, 1982 (42) studied the effect of tea on ionisable iron. The inclusion of one cup of tea (200 ml) in a breakfast reduced the ionisable iron by nearly 50 percent.

Morck *et al*, 1983 (8) examined the inhibitory effect of coffee and tea on food iron absorption. The hamburger meal was used in this study. A cup of coffee, which was prepared by adding 200 ml boiling water to 1.5 g instant coffee, reduced iron absorption from a hamburger meal by 39 percent, while tea that was prepared by 1.75 g instant tea in boiling water 200 ml and steeping for 4 minutes reduced iron absorption by 64 percent.

Merhav *et al*, 1985 (49) evaluated the effect of tea drinking on the occurrence of microcytic anemia in infants. The percentage of tea drinking infants with mycrocytic anemia (32.6%) was significantly higher than that of the non-tea drinkers (3.5%). The daily amount of tea drunk was 50-750 ml. The tea drinkers had significantly lower mean levels of hemoglobin than the non-tea drinkers.

Brown *et al*, 1990 (44) studied the influence of Jamaican herb teas and other polyphenol-containing beverages on dialyzable iron by in vitro method. This study reported the content of total polyphenols in Jamaican herb tea such as orange peel, lime leave and rosemary, was lower than in infusions of black tea, green tea, coffee and cocoa. *In vitro* methodology, when black tea and green tea infusions were added to the cereal-meal reduced dialysable iron by 43 percent and 63 percent, respectively, compared to the water control. Cocoa reduced dialyzable iron by 20 percent compared to the control value.

Garcia-Lopez *et al*, 1990 (50) evaluated the effect of tannin content on iron bioavailability from several legumes. The foods in this study were isolated soy protein, chickpea and red kidney bean containing 4, 3 and 84 mg of tannin (as catechin equivalents) respectively. Retention of iron from a red kidney bean test meal was

increased from 69.3 to 73 percent when about 90 percent of the extractable tannins were removed, but the difference was not statistically significant.

Tuntawiroon *et al*, 1991 (51) examined the effect of the vegetable, Yod Kratin, which contained a considerable amount of iron-binding phenolic compounds on nonheme-iron absorption from a typical Southeast Asian meal. The results showed that when 30 g of Yod Kratin (corresponding to 87.6 mg tannic acid equivalents) was ingested with meal, the percent iron absorption was decreased a 50 percents, while when increasing the ingested Yod Kratin, the percent iron absorption was more decreased.

Reddy *et al*, 1991 (52) reported tea had an inhibitory effect on nonheme iron absorption both in humans and rats. When tea (prepared by adding 1.75 g tea to 200 ml boiling water and steeping for 4 min, and triple-strength tea was prepared similarly with 5.25 g tea) was served with a meal for volunteers, iron absorption was inhibited by 83.5 and 91.5 percent (triple-strength tea). For animal studies the tea was freeze dried and mixed with the meal. In rats, absorption fell from 49.8 to 45.7 percent (inhibited by 8.2%) and a more significant decrease from 62.6 to 44.7 (inhibited by 28.6%) when the amount of tea was increased to triple-strength.

Mehta *et al*, 1992 (53) investigated the association of coffee and tea consumption to anemia. Average daily consumption of 3.7 cups of tea for the normal group was significantly higher than the anemic group but negatively associated with anemic status. Demographic characteristics such as race, education, sex, age and poverty were more important predictions of anemic status than coffee and tea.

South *et al*, 1997 (54) assessed the effect of tea on iron absorption in rats. Consumption of tea with an iron-containing meal significantly reduced iron absorption while tea alone consumption would not affect iron absorption.

Hurrell *et al*, 1999 (55) estimated the effect of different polyphenol-containing beverages on iron absorption from a bread meal in adult human subjects. The test beverages containing different polyphenol structures such as chlorogenic acid in coffee, mononeric flavonoids in herb teas or complex polyphenol polymerization (tannins) in black tea and cocoa. Inhibition by black tea 300 ml was 79 to 94 percent that more inhibited than cocoa and herb teas.

Layrisse *et al*, 2000 (56) determined the bioavailability of amino acid chelated iron (ferrochel) added to fortify breads. Polyphenols present in coffee and tea inhibited iron absorption in a dose-dependent manner. American-type coffee did not modify iron absorption significantly, whereas tea reduced iron absorption from ferrochel by 50 percent.

Samman *et al*, 2001 (57) sought to determine the effect of phenolic-rich extracts obtained from green tea or rosemary on nonheme iron absorption. The iron absorption decreased from 12.1 to 8.9mg in presence of green tea extract and from 7.5 to 6.4 mg in presence of rosemary extract.

Table 3 Summary of the effect of food tannin from previous studies

Researcher	Method	Meal	Food tannin	Result
Disler *et al*, 1975 (6)	*In vivo*	Bread	Tea 200 ml	Reduced 79%
Rossander *et al*, 1979 (46)	*In vivo*	-Wheat roll, margarine, orange, marmalade, cheese	Tea 150 ml	Reduced 56%
		-The same, egg, bacon	Tea 150 ml	Reduced 52%
Kojima *et al*, 1981 (47)	*In vitro*	Cooked pinto bean	Tea	Reduced >50%
			20% ethanol	Slight decrease
			Freshly brewed coffee & instant coffee	Little effect
Hallberg *et al*, 1982 (48)	*In vivo*	Hamburger, string bean, mashed potatoes	Tea 250 ml	Reduced 62%
			Coffee 250 ml	Reduced 35%
Rao *et al*, 1987 (42)	*In vitro*	Wheat	Tea 200 ml	Reduced 50%
Morck *et al*, 1983 (8)	*In vivo*	Hamburger	Tea 200 ml	Reduced 64%
			Coffee 200 ml	Reduced 39%
Merhav *et al*, 1985 (49)	Survey	-	Tea 50-750 ml per day	Tea drinkers had lower mean levels of hemoglobin than non-tea drinkers
Brown *et al*,1990 (44)	*In vitro*	Cereal-milk test meal	Tea infusion 50ml	Reduced 43-63%
			Cocoa infusion 50 ml	Reduced 20%

continue

Researcher	Method	Meal	Food tannin	Result
Garcia-Lopez *et al*, 1990 (50)	*In vitro*	Red kidney bean	Catechin	Not significant
Tuntawiroon *et al*, 1991 (51)	*In vivo*	Rice, fried fish, curry	Yod Kratin 20 g	Reduced 90%
Reddy *et al*, 1991 (52)	*In vivo*	Bun, French fries, milk shake	Tea 200 ml	Reduced 83.5-91.5%
			Freeze dried tea	Reduced
Mehta *et al*, 1992 (53)	Survey (in USA)	-	Coffee & tea	Coffee&tea intake was not a significant predictor of anemia
South *et al*, 1997 (54)	In rats	-	Tea	Reduced
Hurrell *et al*, 1999 (55)	*In vivo*	Bread	Tea 300 ml	Reduced 79-94%
Layrisse *et al*, 2000 (56)	*In vivo*	Corn bread	Tea infusion containing 1.6 g tea leaves	Reduced 50%
			Coffee 2 g	Not significant
			Coffee 4 g	Reduced 50%
Samman *et al*, 2001 (57)	*In vivo*	Bread	Tea extract	Reduced 26 %
			Rosemary extract	Reduced 15%

The results from previous studies of the effect of iron-binding phenolic compounds in foods supported the results of synthetic phenolic compound studies, that the galloyl phenolic group is the structure mainly responsible for the inhibition of iron absorption by phenolic compounds.

Regarding inhibition of nonheme iron absorption by polyphenolic-containing beverages, tea had more inhibitory effect than other beverages such as coffee, cocoa or herb tea. Thus, this study selected tea to study the effect of beverages that contained phenolic compounds on nonheme iron availability from rice-based test meals and to analyze the content of tannic acid in tea, because tannic acid might be the main ingredient containing polyphenol in tea that inhibited iron availability.

Determination of tannic acid equivalents in foods in this study used the method of Brune *et al* (7), because it has been devised to determine specifically the iron-binding properties of phenolic compounds and potential interaction with the absorption of dietary iron.

Tea

Tea is made from the young leaves and unopened leaf buds of the tea plant, Camellia sinensis, a species that includes some very distinct varieties. Tea thrives in a tropical climate and altitudes up to 6,000 feet. Most of the tea used comes from Japan, India, Sri Lanka, and the East Indies, where the climate is appropriate for the cultivation of tea. Although tea requires as much as 68 inches of water per year for good production levels, irrigation is a practical solution for meeting this need. The tea bush is an evergreen and it is ready to yield tea leaves after three years of growth. To maintain a convenient height of three to five feet, this evergreen shrub is pruned continually during its 25 to 50 years of productive life. The leaves are hand plucked from new shoots and about 6,000 leaves are needed to make one pound of manufactured tea (58, 59).

1. Classification of tea

Tea is classified into three groups, depending on the method of processing the leaves. Black tea has gained the widest acceptance in the United States, but the other two types, oolong and green tea, are also available. A brief discussion of processing techniques will reveal the reasons for the different characteristics of the three types. Further subclassification is made within each type according to size of the leaf, variety of tea leaves, area in which it was grown, and flavoring substances added.

1.1 Black tea

The process of drying tea leaves involves relatively few steps. In the case of black tea, these include:

a. Withering the plucked leaves to soften them and partially dry them.

b. Passing the withered leaves under rollers to rupture cell walls and release the enzymes and juices.

c. Fermenting the rolled leaves by exposing them to the air at about 80° F for 2 to 5 hours.

d. Drying or firing the fermented leaves in ovens at about 200°F, which inactivates the enzymes and decreases leaf moisture to about 4 percent.

1.2 Green tea

In the case of green tea the steps involve:

a. Green tea leaves are steamed initially rather than withered in order to inactivate the enzyme that would cause fermentation.

b. Rolling the leaves as above, since rupture of the cell walls also has the beneficial effect of making the leaves easier to extract during subsequent brewing.

c. Drying or firing the leaves.

1.3 Oolong tea

Oolong tea is intermediate between green tea and black tea. The initial processing of oolong tea, a brief fermentation, is done on the tea farms prior to collection for a subsequent firing in the town. Oolong tea has color, flavor and aroma characteristics reminiscent of both black and green teas because of its partical fermentation. Taiwan is particularly noted for the production of fine oolong tea.

2. The chemistry of tea

Tea leaves contain three important kinds of constituents that affect brew quality. These are caffeine, which gives tea is stimulating effect; tannins and related compounds which contribute color and strength, often associated with the terms body and astringency; and essential oils which provide flavor and aroma. Plant variety, geographic location, climate and cultural practices, including processing method, influence the chemical content in tea (59, 60).

3. Preparation of brewed tea

Tea is served in a variety of ways, varying with the country in which it is prepared. A pleasing cup of tea which contains adequate caffeine and a minimum of polyphenols ideally is prepared by using soft water that has been freshly boiled. Freshly boiled water is important because it still contains enough oxygen to give the tea a fresh, pleasant flavor. Very hard water will react with the polyphenols, causing

them to precipitate and form an unattractive film that floats on the surface of the tea and coats the cup.

Several researchers in the past prepared tea from packaged tea bag and/or other preparations (61, 62). From previous studies, that studied the effect of tea on nonheme iron absorption, tea was prepared in different ways such as using different volumes of water, steeping time and the amount of tea leaves (46-49, 53). Poonphonkul showed that the mean value of caffeine and other minerals increased rapidly from 3 to 10 minute brews, thereafter in 20 and 30 minute brews, they were slightly increased. The previous study prepared brewed tea by a practical procedure in Thailand (63). Thus, in this study use similarly prepared brewed tea by using tea leaves 1.5 g and steeping them in boiling water at a temperature of 95°C for 10 minutes.

Assessable method of iron absorption

Assessments of iron absorption from foods were used for estimation of the amount of iron received in man. Iron absorption assessment usually could be divided into three methods e.g. calculation of iron from a meal, analysis of iron absorption in both human being and test animals (*in vivo* method) and in the laboratory (*in vitro* method).

1. Calculation of iron from a meal

There were many methods of quantity study of estimated absorbable iron from a meal but calculation from total iron was a convenient method and could be used for study in a big group of people and used for solving iron deficiency problems. The World Health Organization prefers the calculation of Monsen in 1978 (16) by iron

absorption estimate in each meal according to the amount of iron that depends on the kinds of iron (heme and nonheme iron).

1.1 Heme iron

Aanalysis showed that in different meats, such as pork, chicken, and fish there were similar amounts of heme iron in 40 percent of total iron, and was absorbed by 23 percent. The other 60 percent would be nonheme iron.

1.2 Nonheme iron

Nonheme iron can be got from meat and meat products and also from cereals, eggs, milk, vegetables, and fruits. Nonheme iron absorption depends on other compositions in a meal, such as enhancers (vitamin C and meat) and inhibitors (phytate, fiber and polyphenols). The calculation of nonheme iron absorption in a meal could divide meals into three groups by level of absorption (low, medium and high iron absorption).

Monsen preferred the calculation method for estimating iron absorption in a meal that followed these compositions:

1. Total iron
2. Heme iron
3. Nonheme iron
4. Ascorbic acid
5. Meat, chicken and fish

According to iron store of human subjects were 500 mg by separate composition of each meal, then added together that would be the amount of absorbable iron from

meals per day. Otherwise, the calculation of Monsen did not use inhibitors such as phytate and fiber but preferred that the calculation of iron absorption in a meal contained high inhibitors, the inhibitors should be calculated together.

From the assessment of iron absorption, low absorbable iron happened in only the cereal composed meal, perhaps only 1 to 2 percent, but when that meal had high quality of enhancers, such as meat or ascorbic acid, iron absorption might rise to 20 to 25 percent. In 1988, the Food and Agriculture Organization and the World Health Organization separated meals into three levels according to absorbable iron.

1) Low bioavailability meal is one in which the iron could be absorbed in 5 percent. The meal has only cereals and dose not have any meat or ascorbic acid. Also, the meal had high quality inhibitors such as corn products, bean, and flour that were in the group of developing countries. The low bioavailability meal could change to a higher bioavailability meal when it got enhancers of iron absorption.

2) Intermediate bioavailability meal is one in which the iron could be absorbed 10 percent. Cereals composed the meal and it had enhancers of iron absorption, such as meat or vitamin C.

3) High bioavailability meal is one in which the iron could be absorbed over 15 percent. It has a high proportion of enhancers such as meat or vitamin C. High bioavailability meals are found in groups of developed countries and in people with a high salary. A high bioavilabiltiy meal might drop down to being an intermediate bioavilability meal if that meal had high inhibitors, such as tea or coffee.

In 1997, Kohmeier *et al* (64) studied the amount of iron absorption in Russia, where rice was used for the main composition of the meal. From calculable information of inhibitors, the amount of absorptive iron was reduced in 3 to 4 percent of consumed iron. Thus, absorptive iron assessment should consider any component of the meal, both enhancers and inhibitors.

2. Analysis of iron availability by *in vitro* method

Analysis of iron absorption by *in vitro* method was studied for many years and modified for exact results. The *in vitro* method is an easy method, quick and not expensive. It uses reproduced digestion like in humans by enzymes in the gastrointestinal tract. The amount of iron in soluble iron is measured and used for the amount of absorptive iron. When compared the results with humans it could be used for advantage because it has nearly the same results. The results of absorptive iron analysis by using the *in vitro* method were closely and highly relative (r=0.948) to human studies (65).

In 1969, Jacob and Greenman (66) found available iron in a meal after it had passed digestion with hydrochloric acid and pepsin which was an enzyme in the stomach, but this method could not find the real absorptive iron because that iron was absorbed in the small intestine.

In 1978, Rao and Prabhavathi (67) improved the *in vitro* method by digestion with pepsin-HCl enzyme at pH 1.5, then digestion by pancreatin-bile at pH 7.5, which was the digestive process in the stomach and intestinal lumen. The amount of iron was found from α-α dipyridyl. The iron value in soluble iron had nearly the same value as the absorptive iron in the human body that could be used as absorptive iron.

In 1981, Miller *et al* (68) improved the study by *in vitro* method in a similar study to Narasinga. But this study added pH control two times for dialysis tube digestion and had $NaHCO_3$ solution within the dialysis tube for pH value modification, and passed an ooze process in the dialysis tube that was an absorptive model of the small intestine for nearing the real status in livings. Low molecular weight iron after passing dialysis would have similar value to absorptive iron in creatures to a greater degree than another methods. This method is popular for analysis of iron availability nowadays.

Recently, cell culture has been used extensively as an *in vitro* method to assess human iron bioavailability. Caco-2 cell, a human colon adenocacinoma cell line, have demonstrated numerous morphological and biochemical characteristic of enterocytes. These cells spontaneously brush border and associated enzymes (69). This cell model has been used in a wide variety of nutritional studies (70) and regulation of iron absorption (71, 72) and iron availability studies (73-75). In iron availability assessment, food sample was digested into 2 step by gastrointestinal enzyme, pepsin and pancreatin-bile at pH 2 and 7.5 for 1 and 2 hours, respectively. In the second step of enzyme digestion, sample was incubated in Caco-2 cell model which composed of culture well and costar insert with dialysis membrane for 2 hours. When the intestinal digestion was terminated, the contents of the upper and lower chamber and the dialysis membrane were removed for iron assay.

3. Analysis of iron absorption by *in vivo* method

Several iron absorption studies were chemical studies or used radioiron for study. The *in vivo* method was used both in human being and animals. This method

was credible in iron absorption measurement, because it measured the amount of iron before consumption and measured the iron that was defecated to evaluate the amount of iron absorption. Keeping before and after information completely was difficult for accurate information (76). Moreover, the study in human beings was not easy because of ethical rules, safety, expense and long time. Different humans can absorb iron differently. Study of animals using the *in vivo* method also had problems, like in humans (77,78).

CHAPTER III

MATERIALS AND METHODS

Experimental design

This study was a 3x4 experimental design to study the effect of brewed tea on nonheme iron availability. The different volumes of brewed tea, 0, 100, 200 and 300 ml, were added into all three types of rice-based test meal, polished, mixed and brown rice-based test meals. The study was repeated three times.

Laboratory equipments

1. Hot air-oven
2. pH meter
3. Spectrometer-Spectronic 21
4. Water bath
5. Vortex mixer
6. Homogenizer
7. Analytical balance
8. Hot plate
9. Muffle furnace
10. Desiccator
11. Filter paper No. 1, 4 and 42
12. Glassware: all glassware was soaked overnight in 5% HNO_3 and

thoroughly rinsed several times with deionized water. Deionized water was used throughout this study.

Test meals

The test meals were set into three types depending on the kinds of rice; particularly, polished, mixed and brown rice-based test meals. Each of those was composed the same amount of the same substances related to iron absorption, total iron, crude fiber, vitamin C and tannic acid. The amounts of total iron (5 mg) and crude fiber (5 g) in the test meals were stipulated from recommended mean that Thai people should receive per 1/3 day. Based on the purposive test meal, the amounts and kinds of raw materials in the test meal were formulated (Table 4).

1. Kinds and the contents of raw material

1.1 Rice

Two kinds of rice, brown and polished, were established to three types of rice-based test meal. The polished rice-based test meal used polished rice 60 g, the mixed rice-based test meal used polished rice 30 g mixed with brown rice 30 g, and the brown rice-based test meal used brown rice 60 g.

1.2 Vegetables

The selected vegetables were based on criteria of that made the contents of chemical composition of the test meal, into the recommended quantities, total iron 5 mg and crude fiber 5 g. Each type of rice-based test meal used the same kinds and amount of vegetables, string bean 150 g, kidney bean 100 g and Thai spinach 50 g.

Table 4 The components in raw test meals and their chemical composition calculated from previous data.

Rice-based test meal	Stipulated chemical compositions	Formulated components		Iron[1] (mg)	Crude fiber[1] (g)	Vitamin C[2] (mg)	Tannic acid[2] (mg)	Phytate[3] (mg)
		Rice	Vegetables					
Polished	-total iron 5mg -crude fiber 5 g -vitamin C same as in test meal -tannic acid same as in test meal	Polished 60 g	-string bean (ถั่วฝักยาว) 150 g -kidney bean (ถั่วแขก) 100 g -Thai spinach (ผักโขมไทย) 50 g	5.3	4.3	109.0	84.6	1151.2
Mixed	-total iron 5mg -crude fiber 5 g -vitamin C same as in test meal -tannic acid same as in test meal	Polished 30 g Brown 30 g	-string bean (ถั่วฝักยาว) 150 g -kidney bean (ถั่วแขก) 100 g -Thai spinach (ผักโขมไทย) 50 g	5.6	4.4	109.0	84.6	1367.2
Brown	-total iron 5mg -crude fiber 5 g -vitamin C same as in test meal -tannic acid same as in test meal	Brown 60 g	-string bean (ถั่วฝักยาว) 150 g -kidney bean (ถั่วแขก) 100 g -Thai spinach (ผักโขมไทย) 50 g	5.9	4.5	109.0	84.6	1583.2

[1] The Thai Food Component Table List, 1987 (79)
[2] Sutthiwong N, 1999 (80)
[3] Boontaveeyuwat *et al*, 1990 (33)

Brewed tea

The brewed tea used in this study was prepared from green tea by 1.5 g of dry tea allowed to steep in 100 ml of boiled water for 10 minutes. This green tea (Green tea No. 1, Sam Ma brand) was purchased from a local supermarket in Bangkok.

Methods

1. Experimental methods

1.1 Chosen raw materials i.e. brown rice, polished rice, string bean, kidney bean and Thai spinach, were determined for total iron, crude fiber, vitamin C, tannic acid, phytate and moisture contents (Figure 3).

1.2 Three types of the rice-based test meal were prepared by weight. The constituents of the test meals as mention above were cooked by :

1.2.1 Adding water 120 ml to rice 60 g and cooking

1.2.2 Three kinds of vegetable i.e. string bean 150 g and kidney bean 100 g were boiled for 3 minutes, and Thai spinach 50 g was boiled for 30 seconds.

1.3 The cooked rice and vegetables were blended together until homogeneous and analyzed for total iron, crude fiber, vitamin C, tannic acid, phytate and moisture contents.

1.4 Various volumes of tea, 100, 200 and 300 ml were added into the homogeneous test meals. After adding the brewed tea, the test meals were determined for iron availability by using the *in vitro* method by Kane and Miller (81) as is shown in Figure 4. Method of iron availability assessment was limited to brewed tea added. Since the addition of very high volume of brewed tea will make food sample be so watery that may give the value of iron availability underestimation.

2. Analytical methods

2.1 Total iron: the samples were heated until they became ash described by Basson and Bohmer (82) and total iron in solution was determined by atomic absorption apparatus.

2.2 Crude fiber: the free-fat sample was treated with hot acid, and subsequently boiled with hot alkali. The residue was the crude fiber of the sample (83).

2.3 Vitamin C: fresh and finely blended samples were used in this part. The filtrate of samples with oxalic acid was reacted with 2,4-dinitrophenylhydrazine solution yielding yellow color (84).

2.4 Tannic acid: tannic acid was extracted from food samples by dimethylformamide in acetate buffer. A ferric ammonium sulphate reagent was added and the resulting color was read spectrophotometrically at two wavelengths (7).

2.5 Moisture: samples weighing about 2 g were placed into dry porcelain dish and placed in a preheated electric oven at 105 °C for 2 hours or until they were of constant weight. The porcelain dishes were transferred directly to the desiccator for cooling. The samples were weighed and the loss of weight as water calculated (85).

2.6 Phytate: the filtrate of dry finely blended samples with HNO_3 reacts with ferric ammonium sulphate (86).

2.7 Available iron: the method for determination of iron availability by Kane and Miller. The method involves a two-stages (pepsin and pancreatin) digestion. The amount of iron which passes into a dialysis sac (dialyzable iron) present during stage 2 was used as an indicator of iron availability (81).

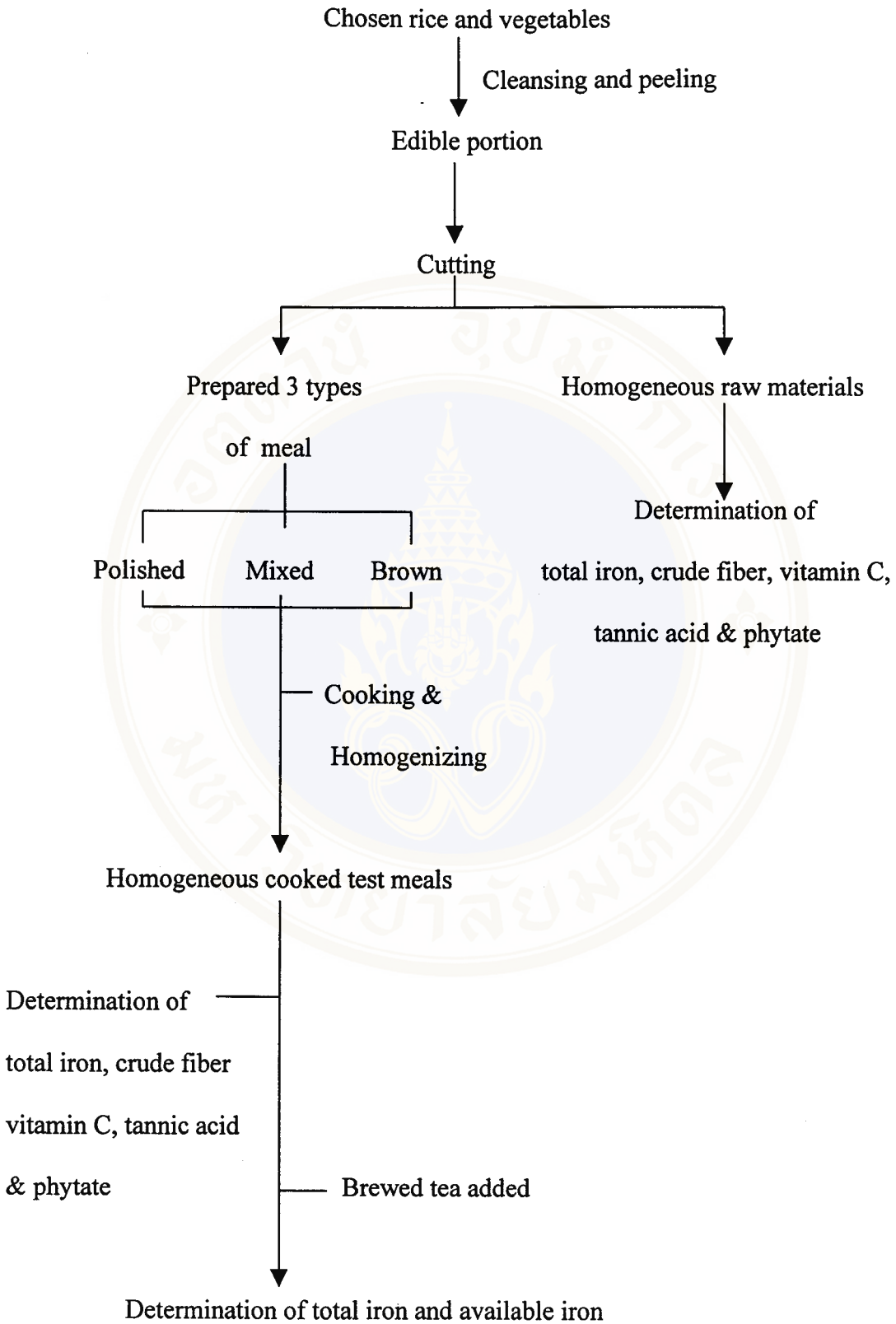


Figure 3 Schematic of experimental method

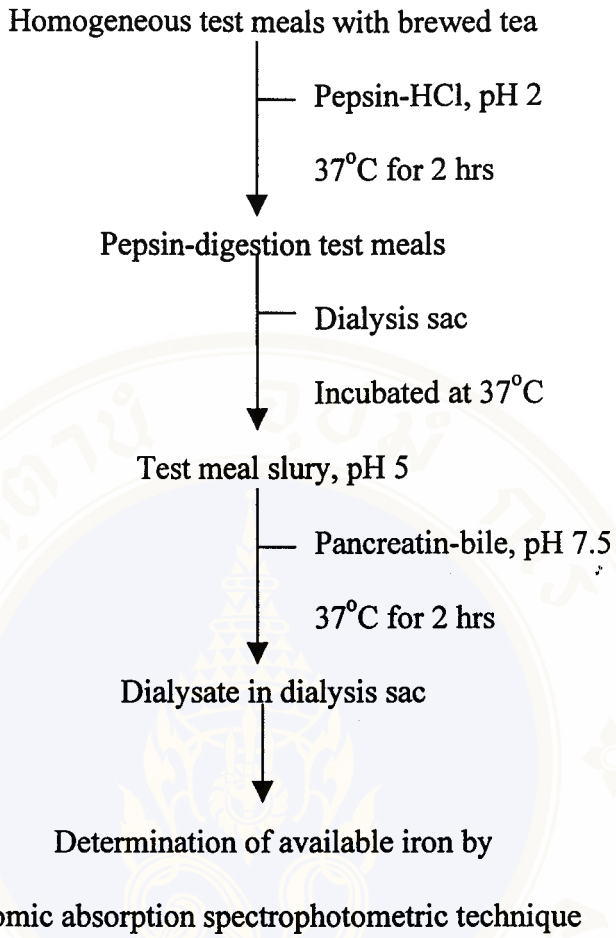


Figure 4 Schematic of determination of available nonheme iron as dialysable iron

Statistical analysis

The non-parametric, Kruskal-Wallis test was used to compare the amount of total iron, crude fiber, vitamin C, tannic acid and phytate in all types of cooked rice-based test meals. Differences of nonheme iron availability in test meals were determined by Kruskal-Wallis test. Mean values for total iron, crude fiber, vitamin C, tannic acid, phytate and available nonheme iron were reported.

CHAPTER IV

RESULTS

Test meals

1. Chemical compositions of raw materials

1.1 Rice

The chemical analysis results of total iron, crude fiber, vitamin C, tannic acid, and phytate in polished and brown rice were shown in Table 5.

Table 5 Chemical analysis of the polished and brown rice used in the study.

Raw materials	Total iron (mg/100g) $\bar{X} \pm SD$	Crude fiber (g/100g) $\bar{X} \pm SD$	Vitamin C (mg/100g) $\bar{X} \pm SD$	Tannic acid equivalents (mg/100g) $\bar{X} \pm SD$	Phytate (mg/100g) $\bar{X} \pm SD$
Brown rice (ข้าวกล้อง)	2.2±0.5	0.7±0.1	Trace	5.78±0.0	1152.90±2.51
Polished rice (ข้าวเจ้าขัดสี)	0.4±0.1	0.4±0.2	Trace	5.69±0.0	480.38±1.06

The results showed that the quantity of total iron in the brown rice was 2.2 mg/100 g while that of the polished rice was only 0.4 mg, approximately 6 times less than that in brown rice. The amount of crude fiber in the brown rice was about 2 times more than in the polished rice (brown rice 0.7 g./100 g, polished rice 0.4 g/100 g). The amounts of vitamin C and tannic acid in the two rice were the same in small amount. The amounts of tannic acid in the brown and polished rice were 5.78 mg/100 g and 5.69 mg/100 g, respectively. The amount of phytate in the polished rice was only

480.38 mg/100 g, which was less than in the brown rice by about 3 times (1152.90 mg/100 g).

1.2 Vegetables

The vegetables, string bean, kidney bean, and Thai spinach were analyzed for chemical composition. The quantities of total iron, crude fiber, vitamin C, tannic acid, and phytate were shown in Table 6.

Table 6 Chemical analysis of the selected vegetables

Raw materials	Total iron (mg/100g) $\overline{X} \pm SD$	Crude fiber (g/100g) $\overline{X} \pm SD$	Vitamin C (mg/100g) $\overline{X} \pm SD$	Tannic acid equivalents (mg/100g) $\overline{X} \pm SD$	Phytate (mg/100g) $\overline{X} \pm SD$
String bean (ถั่วฝักยาว)	0.7±0.1	1.4±0.2	41.23±4.2	41.65±8.5	280.48±8.61
Kidney bean (ถั่วแขก)	1.64±0.1	1.3±0.1	35.43±0.6	3.30±5.2	237.11±4.28
Thai Spinach (ผักโขมไทย)	2.20±0.2	1.0±0.3	121.40±2.7	30.10±3.8	258.64±6.79

The amounts of total iron in three kinds of vegetable were arranged in order to amount from small to large: string bean 0.7 mg, kidney bean 1.64 mg, and Thai spinach 2.2 mg per 100 g of fresh vegetables. The amounts of crude fiber in string bean and kidney bean were approximately 1.4 g, but Thai spinach had crude fiber 1.0 g. In these three kinds of vegetable, Thai spinach had the largest amount of vitamin C, 121.40 mg, more than string bean (41.23 mg) and kidney bean (35.43 mg) by approximately 3 times. The string bean had tannic acid 41.65 mg, which was more than the kidney bean (3.30 mg) and Thai spinach (30.10 mg). The amounts of phytate

in these three vegetables were not difference different; the string bean, kidney bean and Thai spinach had 280.48, 237.11 and 258.64 mg/100 g of phytate, respectively.

Regarding to the chemical contents between the rice group and the vegetable group, the rice group had more phytate than the vegetable group, especially the phytate of the brown rice was almost 4 times more than the vegetable group, whereas the vegetable group had more vitamin C than the rice group. In addition, these three vegetables had more crude fiber than the rice group.

2. Chemical compositions of the test meal.

All types of rice-based test meal were composed of the same kind and amount of vegetable, but there were 3 different types of rice. The string bean 150 g, kidney bean 100 g and Thai spinach 50 g were used in all types of rice-based test meals. The polished rice 60 g was used in the polished rice-based test meal, mixed polished rice 30 g and brown rice 30 g was used in the mixed rice-based test meal, and brown rice 60 g was used in the brown rice-based test meal. The analysis of chemical composition was done in both uncooked and cooked test meals.

2.1 Chemical compositions of the uncooked test meal.

The analysis data of the uncooked test meals are shown in Table 7. The amount of total iron in the polished rice-based test meal (4.03 mg) was less than those in the mixed (4.46 mg) and brown rice-based test meals (4.89 mg). Regarding the amount of crude fiber, the polished, mixed and brown rice-based test meal had crude fiber 4.14, 4.23, and 4.35 g/meal, respectively. The amount of vitamin C and tannic

Table 7 The chemical compositions in the uncooked test meals determined by chemical analysis.

Rice-based test meal	Stipulated chemical compositions	Components		Iron (mg)	Crude fiber (g)	Vitamin C (mg)	Tannic acid (mg)	Phytate (mg)
		Rice	Vegetables					
Polished	-total iron 5mg -crude fiber 5 g -vitamin C same as in test meal -tannic acid same as in test meal	Polished 60 g	-string bean (ถั่วฝักยาว) 150 g -kidney bean (ถั่วแขก) 100 g -spinach (ผักโขมไทย) 50 g	4.03±0.3	4.14±0.5	157.98±2.5	84.24±0.2	1075.00±10.3
Mixed	-total iron 5mg -crude fiber 5 g -vitamin C same as in test meal -tannic acid same as in test meal	Polished 30 g Brown 30 g	-string bean (ถั่วฝักยาว) 150 g -kidney bean (ถั่วแขก) 100 g -spinach (ผักโขมไทย) 50 g	4.46±0.3	4.23±0.5	157.98±2.5	84.27±0.2	1277.13±10.3
Brown	-total iron 5mg -crude fiber 5 g -vitamin C same as in test meal -tannic acid same as in test meal	Brown 60 g	-string bean (ถั่วฝักยาว) 150 g -kidney bean (ถั่วแขก) 100 g -spinach (ผักโขมไทย) 50 g	4.89±0.3	4.32±0.5	157.98±2.5	84.29±0.2	1478.89±10.3

acid in all types of rice-based test meal were approximately 157.98 mg and 84.24 mg/meal. The difference of the phytate content in each type of rice-based test meal was approximately 200 mg. The polished rice-based test meal had the least phytate, 1075 mg, of all three groups. The mixed rice-based test meal had 1277.13 mg of phytate, that was less than the brown rice-based test meal. In conclusion, the brown rice-based test meal had the most phytate content (1478.89 mg).

2.2 Chemical compositions of the cooked test meal

The uncooked test meals were cooked by a following the method as described in Chapter 3. The cooked rice and vegetables were homogenized. Analysis of the chemical compositions in the test meals (Table 8) showed that the amount of total iron in the brown rice-based test meal was 4.23 mg/meal, and 4.06 mg/meal in the polished rice-based test meal. Considering to the amount of crude fiber, the brown rice-based test meal had the highest crude fiber content, the polished rice-based test meal had the lowest, but the content was close to each other (in the brown rice 4.30 g, mixed rice 4.25 g and polished rice 4.10 g/meal, respectively). The amount of vitamin C and tannic acid in all three cooked test meals was about 132 mg of vitamin C and about 83 mg of tannic acid. The Kruskal-Wallis test was used for comparing the quantity of total iron, crude fiber, vitamin C, tannic acid in three cooked test meals, the results showed no significant difference ($p>0.05$). Only analysis data of phytate had statistically significant. When compared by multiple comparison, the mean difference of phytate content was significant at the 0.001 level. Concerning the difference in amounts of phytate from three cooked test meals, the polished rice-based test meal was differed from the mixed rice-based test meal in phytate by 202.18 mg. The polished

Table 8 Chemical compositions of the cooked test meals

Rice-based test meal	Weight (g) $\bar{X}$±SD	Iron (mg) $\bar{X}$±SD	Crude fiber (g) $\bar{X}$±SD	Vitamin C (mg) $\bar{X}$±SD	Tannic acid (mg) $\bar{X}$±SD	Phytate (mg) $\bar{X}$±SD
Polished	440.98±3.90	4.06±0.5	4.10±0.2	133.48±13.2	83.29±1.8	1075.00±19.8*
Mixed	443.66±4.76	4.11±0.9	4.25±0.2	133.85±12.3	83.48±1.1	1277.18±19.0*
Brown	442.10±5.40	4.23±0.4	4.30±0.2	137.23±8.05	83.73±2.08	1479.03±23.7*
χ^2 Kruskal	0.356	4.667	4.528	0.389	1.167	31.5
p value	0.837	0.558	0.542	0.823	0.097	0.000

* The mean differences of phytate content between each types of the test meal were significant at the 0.001 level

rice-based test meal had a difference in phytate from the brown rice-based test meal of 404.03 mg. The mixed rice-based test meal had a difference in phytate from the brown rice-based test meal of 201.85 mg.

The total iron and tannic acid contents in the brewed tea

Tea leaves 1.5 g were steeped in 100 ml of boiling distilled water for 10 minutes. The brewed tea was analyzed for total iron, tannic acid and iron availability. The results were shown in Table 9.

Table 9 The total iron and tannic acid contents in the brewed tea 100 ml.

Item	Content $\bar{X}$±SD
Total iron (mg)	0.1229±0.0
Tannic acid (mg)	46.22±0.25
Available iron (mg)	0.0303±0.0
% of available iron : total iron	24.98±0.6

In the brewed tea 100 ml, the amounts of total iron, tannic acid and available iron were 0.1229, 46.22 and 0.0303 mg, respectively. When available iron was calculated and made into percentage by comparison with total iron, it was 24.98%.

Thus, when the volumes of brewed tea adding in the cooked test meal was increased from 100 ml to 200 and 300 ml, it consequently made an increase of total iron and tannic acid 2 and 3 times as well. In the brewed tea 200 ml, there would be total iron 0.2458 mg and tannic acid 92.44 mg, and then the rewed tea 300 ml would have total iron of 0.3687 mg and tannic acid 138.66 mg (Table 10).

Table 10 The total iron and tannic acid contents in the brewed tea by adding volume

Volume of brewing tea (ml)	Total iron (mg)	Tannic acid (mg)
0	0	0
100	0.1229	46.22
200	0.2458	92.44
300	0.3687	138.66

The total iron and tannic acid contents in the test meal after added brewed tea

After preparing the homoginized test meals, various volumes of brewed tea, 100, 200 and 300 ml, were added to the test meal. Three types of rice-based test meal had 83.29, 83.46 and 83.78 mg of tannic acid/meal (Table 11). Thus, after adding brewed tea to each test meal, these would be an increase in tannic acid of 46.22 mg and of iron 0.1229 mg in each 100 ml of brewed tea added. The amount of tannic acid and iron in the test meal after adding brewed tea was used to calculate the ratio. At a volume of brewed tea of 0 ml, the polished, mixed and brown rice-based test meal had ratios between tannic acid and total iron of 20.5:1, 20.3:1 and 20.0:1, respectively. If we compared the ratio of tannic acid to total iron in the same type of test meal but with different volumes of brewed tea added, it showed that the ratio of tannic acid to total iron was higher when the volume of brewed tea added increased. These ratios were of a similar pattern in all three categories of rice-based test meal with adding the brewed tea.

Table 11 Ratio of tannic acid and total iron in the cooked test meal when brewed tea was added

Rice-based test meal	Volume of Brewed tea (ml)	Tannic acid (mg)	Total Fe (mg)	Tannic acid:Total iron
Polished	0	83.29	4.0571	20.5:1
	100	129.51	4.1800	31.0:1
	200	175.33	4.3029	40.7:1
	300	221.95	4.4258	50.1:1
Mixed	0	83.46	4.1084	20.3:1
	100	129.68	4.2313	30.6:1
	200	175.90	4.3542	40.4:1
	300	222.12	4.4771	49.6:1
Brown	0	83.78	4.2275	20.0:1
	100	130.00	4.3504	29.9:1
	200	176.22	4.4733	39.4:1
	300	222.44	4.5962	48.4:1

Comparison between the proportion of iron from tea to total iron in the test meal with brewed tea (Table 12), the percent iron from tea were 2.94, 5.71 and 8.33 of total iron in the polished rice-based test meal with adding 100, 200 and 300 ml of brewed tea, respectively. The values were of 2.90, 5.65 and 8.24 percent in the mixed rice-based test meal. For the brown rice-based test meal, they were 2.83, 5.49 and 8.02

percent. The percentage of brewed tea iron among the three types of test meal were nearly equal values at the same brewed tea volume.

Table 12 Percentages of tea iron per test meal iron in the test meals with brewed tea.

Rice-based test meal	Volume of brewed tea (ml)	Test meal iron (mg)	Tea iron (mg)	Total iron (mg)	Tea iron (% total iron)
Polished	0		0	4.0571	0
	100	4.0571	0.1229	4.1800	2.94
	200		0.2458	4.3029	5.71
	300		0.3687	4.4258	8.33
Mixed	0		0	4.1084	0
	100	4.1084	0.1229	4.2313	2.90
	200		0.2458	4.3542	5.65
	300		0.3687	4.4771	8.24
Brown	0		0	4.2275	0
	100	4.2275	0.1229	4.3504	2.83
	200		0.2458	4.4733	5.49
	300		0.3687	4.5962	8.02

Availability of iron from rice-based test meal

After adding the brewed tea to the test meals, they were analyzed for iron availability by the *in vitro* method. The test meal were digested by enzymes in 2 steps and passed into the dialysis sac. In the final step, dialysates (the solution in the dialysis sac) were analyzed for available iron by atomic absorption analysis. Values of available iron from the test meals were shown in Table 13.

Table 13 Iron availability from the cooked test meals consisting of different volumes of brewed tea

Rice-based test meal	Volume of brewed tea (ml)	Available iron (mg) $\bar{X}$±SD
Polished	0	0.3870±0.03
	100	0.2836±0.07
	200	0.3356±0.07
	300	0.3758±0.11
Mixed	0	0.3647±0.04
	100	0.2854±0.01
	200	0.3603±0.01
	300	0.3608±0.07
Brown	0	0.3589±0.04
	100	0.3017±0.02
	200	0.3339±0.05
	300	0.3490±0.05

Regarding the availability of iron from the test meals with 0 ml brewed tea (control), the polished rice-based test meal (0.3870 mg) had more available iron than the mixed rice-based test meal (0.3647 mg) and brown rice-based test meal (0.3589 mg); these values were calculated as 9.8862, 8.8170 and 8.6544 of total iron, respectively (Table 14). When added brewed tea 100, 200 and 300 ml into test meal, it increased the ratio of tannic acid to iron in all types of test meal from 20:1 to 30:1,

Table 14 Percent iron availability and inhibition of brewed tea from the cooked test meals.

Rice-based test meal	Volume of brewed tea (ml)	Ratio of tannic acid:total iron	Iron availability (%)	Iron availability reduction (%)	χ^2	P-value
Polished	0	20.5:1	9.8862±0.02	-	1.5	p>0.5
	100	30.0:1	7.2531±0.02	27		
	200	40.7:1	8.5114±0.02	14		
	300	50.1:1	9.5152±0.04	4		
Mixed	0	20.3:1	8.8170±0.02	-	2.472	p>0.25
	100	30.6:1	7.2241±0.00	18		
	200	40.1:1	7.8959±0.01	10		
	300	49.6:1	8.4310±0.01	4		
Brown	0	20.0:1	8.6544±0.02	-	2.18	p>0.5
	100	29.9:1	7.1746±0.01	17		
	200	39.4:1	7.8484±0.01	9		
	300	48.4:1	8.1540±0.01	6		

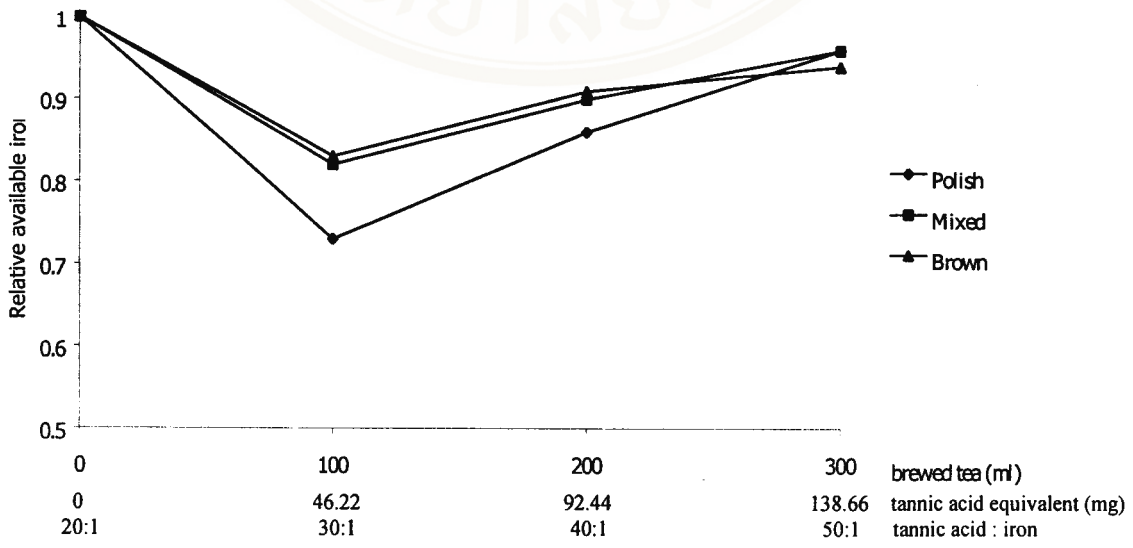


Figure 5 The relative available iron in the test meals by brewed tea volume and tannic acid equivalent

40:1 and 50:1, respectively. The addition of 100 ml of brewed tea into three types of rice-based test meal made percent iron availability from the polished rice-based test meal decreased to 0.1034 mg (27% iron availability reduction). The mixed rice-based test meal decreased by 18 percent, and the brown rice-based test meal decreased by 17 percent. When brewed tea 200 and 300 ml in each type of meal, iron availability slightly increased with increasing volume of adding brewed tea. These were the iron availability of meals with brewed tea 200 ml was higher than the meals with brewed tea 100 ml, and the iron availability with brewed tea 300 ml was higher than the meals with brewed tea 100 and 200 ml, but not higher than the test meal without brewed tea (Figure 5).

Table 15 Comparison of the mean of percent iron availability in 3 types of rice-based test meal with the same volume of brewed tea

Volume of brewed tea (ml)	% Iron availability in rice-based test meals			χ^2	P-value
	Polished	Mixed	Brown		
0	9.8862±0.02	8.8170±0.02	8.6544±0.02	1.805	p>0.05
100	7.2531±0.02	7.2241±0.00	7.1746±0.01	0	p>0.05
200	8.5114±0.02	7.8959±0.01	7.8484±0.01	0.18	p>0.05
300	9.5152±0.04	8.4310±0.01	8.1540±0.01	1.5	p>0.05

However, the different values of iron availability from the same type of rice-based test meal with different brewed tea added were not statistically significant by Kruskal-Wallis test (p>0.05). Considering the iron availability from three types of

rice-based test meal at the same volume of brewed tea, it was found that iron availability from polished rice-based test meal was higher than those from mixed and brown rice-based test meals, but those were not significant difference ($p>0.05$) (Table 15).

The effect of brewed tea on iron availability in the present study was compared with the other previous studies. This comparison had a purpose for finding the difference between the present and previous studies that might be the cause of the different result. It probably due to the type of meal, type of tea and preparation of brewed tea, especially analytical method of iron availability. However, there was no study of adding brewed tea, 100 ml, to study the inhibitory effect on nonheme iron absorption (or availability). The results of the comparison were shown in Table 16.

After compared the present study with the previous studies at the same volume of brewed tea, there found the main difference that might strongly affect the inhibitory effect on iron availability as the concentration of brewed tea. The different preparation of brewed tea such as the amount of tea, steeping time and type of tea, all of these might have effect on the concentration of tannic acid in brewed tea. Thus, the ratio of tannic acid to iron was considered instead of the brewed tea volume. The comparison of tannic acid to iron and percent reduction of iron availability between the present study and other previous studies were shown in Table 17

Table 16 Comparison between the effect of brewed tea volume on iron availability in the present study and the previous studies.

Volume of brewed tea (ml)	Tea	Meal	Iron reduction (%)	Method	Reference
100	Green tea leaves 1.5 g, 10 min	Rice and vegetables	27, 18, 17*	*In vitro*	The present study
200	Green tea leave 3.0 g, 10 min	Rice and vegetables	14, 10, 9*	*In vitro*	The present study
	Tea 5 g	White bread with synthetic iron (ferric chloride)	68	*In vivo*	Disler *et al*, 1975 (7)
	Instant black tea 1.75 g, 4 min	Hamburger (beef patty and bun)	64	*In vivo*	Morck *et al*, 1983 (8)
300	Green tea leaves 4.5 g, 10 min	Rice and vegetables	4, 4, 6*	*In vitro*	The present study
	Black tea 3 g, 10 min	Bread	79	*In vivo*	Hurrell *et al*, 1999 (55)

* Percent of iron availability reduction from polished, mixed and brown rice-based test meals, respectively

Table 17 Comparison of the effect of ratio between tannic acid and iron on iron availability in the present study and the previous studies.

Meal	Source of tannic acid	Amount of tannic acid (mg)	Tannic acid: iron	Percent reduction	References
Rice and vegetables	Tea	83.51*	20.3:1	-	The present study
		129.73*	30.5:1	27, 18, 17	
		175.82*	40.2:1	14, 10, 9	
		222.17*	49.4:1	4, 4, 6	
Free phytate-vitamin C bread	Synthetic tannic acid	5	1.3:1	20	Brune *et al.*, 1989 (7)
		10	2.6:1	40	
		25	6.6:1	67	
		50	13.2:1	86	
		100	26.3:1	88	
		200	52.6:1	94	
White tannic acid-free bread	Synthetic tannic acid	12	2.4:1	34	Siegenberg *et al.*, 1991 (45)
		26	5.2:1	55	
		55	11:1	73	
		263	53:1	82	
		833	166:1	81	
Rice, fried fish, a curry (Nam Prik Kapi)	Yod Kratin	87.6*	11:1	50	Tuntawiroon *et al.*, 1991 (51)
		146*	28:1	73	
		292*	48:1	81.25	
		438*	79:1	84.4	
		584*	101:1	86.7	

* mg of tannic acid equivalents

CHAPTER V

DISCUSSION

The effect of brewed tea on nonheme iron absorption has been extensively investigated. Most previous researchers studied the effect of brewed tea on nonheme iron absorption in Western type diets. Western type diets are mostly composed of bread and meat, while Asian diets, including Thailand, are mainly composed of rice and vegetables. The present study demonstrated the effect of brewed tea on nonheme iron availability from different types of rice-based test meal when different volumes of brewed tea, 0,100, 200 and 300 ml were added.

Test meals

The present study investigated the availability of nonheme iron in ferric form (Fe^{3+}). Thus, raw materials were selected from plant sources i.e. rice and vegetables for setting test meals. The test meals were composed of three kinds of rice: polished, mixed and brown rice-based test meals. The polished rice-based test meal used 60 g polished rice, the mixed rice-based test meal used a mixture of polished and brown rice at a ratio of 1:1 (60 g), and the brown rice-based test meal used 60 g brown rice. To each test meal were added the same kinds and amounts of vegetable, particularly string bean 150 g, kidney bean 100 g and Thai spinach 50 g.

1. Chemical compositions of raw material

1.1 Rice

A common meal in Thailand is composed of rice. In urban areas, polished

rice is the most commonly eaten, whereas most people in rural areas consume brown rice (87). The 1998 remarks of His Majesty the King of Thailand, King Bhumibhol, to the mass media about consuming brown rice made Thai people interested in consuming brown rice more than in the past. In addition, people who care for their health will consume brown rice because brown rice has more vitamins and minerals than polished rice (88). This reason made brown rice more favored than in the past. Some people begin to consume brown rice but are not familiar with the taste and often mix it with polished rice. The present study used Thai Hom Mali rice because, after being cooked it was soft and attractive to consume. Two kinds of this rice, polished and brown rice, were used in the present study because of the above reasons.

Brown rice had more total iron, crude fiber and phytate than polished rice, approximately 5.5, 2 and 2.5-fold, respectively. The content of total iron in brown rice was closed to the estimated value from the food table (2.8 mg/100 g), whereas the content of total iron from polished rice was less than the estimated value from the food table (1.9 mg/100g), approximately 5-fold. Brown and polished rice had crude fiber content were quite equal to the estimated value from the food table, and also had a phytate content was similar to that from the previous study of Boontaveeyuwat (33). Vitamin C and tannic acid contents of brown rice was closed to that from polished rice (vitamin C contents were trace, tannic acid contents were approximately 5.70 mg/100 g).

1.2 Vegetables

Because kinds and amounts of vegetable were variable factors of total iron and crude fiber contents in the studied test meals, the selected vegetables were those

that made the chemical compositions into the recommended quantities, total iron 5 mg and crude fiber 5 g. Apart from the chemical composition of these vegetables, the decision on selecting vegetables also depended on those that could be bought easily, and were available in all seasons and not expensive.

On the first time of selecting vegetables, Yod Kratin 100 g and sting bean 150 g were used in the test meal because these made the content of total iron and crude fiber up to the recommended quantity. But the using of Yod Kratin was limited by the season because Yod Kratin was available only in the rainy season. Thus, other vegetables were selected instead of Yod Kratin. These vegetables were string bean 150 g, kidney bean 100 g and Thai spinach 50 g.

The amount of total iron in Thai spinach was higher than in the string bean and kidney bean but total iron in the string bean was nearly equal to the content estimated from the food table, but the kidney bean and Thai spinach had less total iron than that from estimation using the food table. All of the vegetables which were used in the test meal had the same amount of crude fiber when compared with the food table. The string bean and kidney bean had less vitamin C than the value from the food table approximately 2-fold, whereas Thai spinach was near the value from the food table. The kidney bean had less tannic acid than the string bean and Thai spinach by approximately 13 and 9-fold, respectively. The phytate content in the 3 kinds of vegetable were different, but not much.

2. Chemical compositions of the test meal

Analysis data of iron in the test meals showed that it was 4-5 mg/meal, which was a little less than the calculation from the Thai food compositions table (5-6 mg). The

difference of total iron content might be the result of raw materials coming from different sources and seasons. The brown rice-based test meal had more total iron than the mixed and polished rice-based test meals. However, the total iron content in all types of rice-based test meal were similar, still near with 1/3 of the recommended quantity that the body should receive per day (women require 15 mg/day total iron). The dietary consumption survey in Thailand (89) showed that the total dietary iron intake was 11.8±4.2 mg/day. Thus, the total iron content in this test meal was close to the dietary consumption survey in Thailand (89).

The crude fiber in the cooked test meal was similar to the content of the raw test meal. These contents were approximately 4 g, which was near the 5 g set content (1/3 of the recommended quantity the body should receive per day, 15-20 g/day) (13), or 12 g per 1/3 day or 36 g per dietary day. Phuwasatein (90) reported Thai men received on average 45±4 g of crude fiber per day, and Thai women received 26±2 g per day. The test meal in the present study had high crude fiber.

The vitamin C content of the test meals both raw materials and cooked test meals, were analyzed. Vitamin C in the uncooked test meal was considerably higher than that in the cooked test meal, which was approximately 157.89 mg and 135 mg in the cooked test meal. Moreover, both vitamin C contents were substantially different from the estimation using the food table, which was 109 mg. A Thai dietary consumption study (89) showed that Thai people consumed on average 94.8 mg of vitamin C per day. The recommended quantity of vitamin C by the Health Department of Thailand was 60 mg per day. Many previous studies reported vitamin C as good for health and that the daily intake of vitamin C should be from 100-200 mg

to 500 mg (91). Thus, the vitamin C content of this test meal was in line with the recommended levels.

The different content of vitamin C in the present study and from that estimated by data from the food table might be results of differences of season and the soil in which the foods were grown. The differences in vitamin C in the uncooked and cooked test meals were the result of the cooking process, because vitamin C can easily be destroyed by heat. In the pasturization process, 25 percent vitamin C is lost and in the sterilization process 60 percent is lost. Up to 100 percent of vitamin C in UHT milk kept for more than 3 months could be lost. Loss of vitamin C is often found in the cooking process when foods are kept for a long time. Keeping vegetables and fruits in a hot place could make them lose vitamin C and the loss of vitamin C between processes depends on the characteristics of the process, the kind of process and the contact of the surface of vegetables and fruits with oxygen (92).

The contents of analytical tannic acid equivalent in the uncooked and cooked test meals were approximately 85 mg, which was close to the content in the three types of rice-based test meal. Tannic acid content was used for information about the test meal. In earlier reviews, very little was known about the tannic acid content of foods, but most studies studied the contents of total polyphenol and tannin. Rao *et al*, (42) determined the tannin content of Indian food. They found rice (*Oryza sativa*) had no tannins and kidney bean (*Dolichops lablab*) had 1024 mg tannin per 100 g. Diets consumed in different parts of India had tannin contents ranging from 1.5 to 2.5 mg per day. In Thailand, only one piece of research determined the tannic acid content in food, but only in one food, Yod Kratin. The smallest amount of Yod Kratin (3 g) had approximately 88 g of tannic acid.

There has been little study of the phytate content of foods, especially in Thailand. Boontaveeyuwat (33) reported high phytate content in pulses, nuts, dry grains and also seeds that could be bred. Rice for use in this study was one kind of dry grain. In the present study, brown rice had more phytate than polished rice, approximately 5-fold. When these were brought together to set up the test meal, the test meal containing brown rice had a higher phytate content than the test meal without brown rice. Thus, the brown rice-based test meal in the present study had more phytate than the mixed and polished rice-based test meals (400 and 200 mg, respectively), and the mixed rice-based test meal had more phytate than the polished rice-based test meal (200 mg).

When the chemical compositions of three types of rice-based test meal were compared by Kruskal-Wallis test, it was found that all types of rice-based test meal had no significant difference in the amount of total iron, crude fiber, vitamin C and tannic acid ($p>0.05$), except that the phytate content in each rice-based test meal had a significant difference ($p<0.001$). The difference was due to the different phytate contents of each rice.

The total iron and tannic acid contents in the brewed tea

1. The total iron content in the brewed tea

There has been little study of total iron in brewed tea. The research of Nagaprateap (93), who analyzed the trace element content of Assam tea leaves grown in Chiangmai, found this tea had a total iron content of approximately 155-245 mg/kg dry weight. Stagg and Millin (94) found black tea had a total iron content of 56-333 mg/kg, and that by consuming 5-6 cups brewed tea there would be an intake of total iron less than 0.02 mg. Poonphunkul (63) studied the content of total iron in brewed

tea that was prepared by using 3 g of tea leaves steeped in 200 ml boiled water for 5 minutes; this brewed tea had total iron of 28.70×10^{-3} mg/100 ml. In the present study, the total iron content in brewed tea was 0.1229 mg/100 ml, which was more than the total iron in brewed tea of the previous studies. However, it was difficult to compare the content of total iron in brewed tea in this study and previous studies because brewed tea in each study was prepared using different methods, and different types and kinds of tea.

The consumed iron content did not depend only on the iron content of the tea, but it depended on the iron content of the water that was used to brew the tea. Gillies *et al*, (95) determined concentrations of minerals in brewed tea. They found that using tap water for preparing brewed tea made the iron content high. The tap water that was used in the study of Gillies *et al*, had approximately 0.11 mg/liter of iron. In the present study, double distilled water (deionized water) was used to brew the tea. Thus, the iron content in the brewed tea was iron from the tea only.

2. The tannic acid content in the brewed tea

Tannic acid from adding various volumes of brewed tea, 100, 200 and 300 ml, were approximately 46.22 mg, and increased 2 and 3-fold, respectively. Rarely had there been any study of the tannic acid content of brewed tea; most studies analyzed the content of total polyphenol. Bravo (39) demonstrated that 100 ml of brewed tea had 75-100 mg total polyphenol. Rao *et al*, (42) studied consuming diets in India, and found brewed tea had tannins of 98 mg/100 ml. The tannic acid content in brewed tea of the present study was less than the content of total polyphenol or tannins from other

previous studies because tannic acid is one kind of tannin, and tannin is one kind of total polyphenol.

In conclusion, the content of iron and soluble polyphenols varied according to the variety of tea, its geographical origin, environmental conditions, agronomic situation and different analytical methods.

Availability of iron from rice-based test meal

The objective of the present study was to investigate the iron availability from different types of rice-based test meal when brewed tea was added in different volumes. Consideration of iron availability was divided into 2 parts. The first consideration was the availability of iron from the same type of rice-based test meal when different volumes of brewed tea were added, and the second consideration was the availability of iron from the different types of rice-based test meal but which had the same volume of brewed tea.

1. Iron availability from the same type of rice-based test meal with various volumes of brewed tea added

The first consideration of the present study is the relationship between different volumes of brewed tea added to the test meal and the degree of inhibition of iron availability. When brewed tea was added to the test meal, iron availability decreased at all levels of brewed tea volume, but the degree of inhibition by increasing brewed tea volume did not increase. The finding were not followed as expectation, and were unlike other previous studies. An attempt to explanation of the finding was shown by 2 main possible reason as belows

1.1 Type of test meal and concentration of brewed tea.

The first attempt to explanation the unexpected results was comparison of the inhibitory effect of brewed tea on iron availability between the present study and other previous study by comparing volume of the brewed tea used. Other previous studies studied the effect of brewed tea on nonheme iron bioavailability by using the same volume of brewed tea as this study, 200 and 300 ml. But there is no study of adding brewed tea, 100 ml, to study the inhibitory effect on nonheme iron bioavailability. Main possible reasons that made the result of the present study unlike other studies were shown when compared one by one between the present study and the other previous studies as in the followings.

At 200 ml of brewed tea, the first previous study that used the same volume as the present study was the study of Disler *et al* (6). Disler *et al*, 1975, studied the effect of 200 ml brewed tea on iron absorption in a study with human volunteers. Brewed tea inhibited the absorption of iron from a bread meal by 68 percent. The reductions iron availability by brewed tea in the present study were 14, 10 and 9 percent in polished, mixed and brown rice-based test meal, respectively, and these results were markedly less than the result of Disler *et al*. Considering study design of the studies, there found 3 points of difference. They are iron forms, test meal, brewed tea and the assessment method of available iron. These differences might affect the results. The test meal in the study of Disler *et al,* was white bread (using 70-80% extraction flour) and sufficient ferric chloride ($FeCl_3$) was mixed into the test meal. The volunteers ate a bread meal without butter or jam. The first point, synthetic iron is free inorganic iron which more easily bind to inhibitors, consequently being less

available to absorption than dietary iron which is organic iron. The second point, other chemical components in the test meal might be factors to make the inhibition by brewed tea lower than the previous study. In the present study, the test meal composed of rice and vegetables, and these had other chemical components such as vitamin C or other minerals and vitamins, which were enhancers of iron availability. The third point that may have had an effect on iron availability might be the concentration of brewed tea. The concentration of brewed tea in the present study was lower than that in the study of Disler *et al.* The brewed tea in the study of Disler *et al.* was prepared from 5 g of tea to 200 ml of water, but the present study used 3 g of tea to 200 ml of water. In addition, the source and quality of tea might be factors that made the effect of brewed tea on iron availability different. The last factor was the assessment of iron available iron because the different method would give the different value.

Another previous study that studied the effect of 200 ml of brewed tea was study of Morck *et al.* In 1983, Morck *et al* (8) evaluated the effect of brewed tea on nonheme iron absorption from a hamburger meal that was composed of a beef patty and a bun. They found tea could inhibit iron absorption more (by 64%) than that of this study by approximately 6-fold. When considering the points of difference in the 2 studies, the main point that might be the cause of different results was the kind of tea in the study. Morck *et al* used instant tea (1.75 g to 200 ml water) but the present study used tea leaves (3 g to 200 ml water). Instant tea is tea that is ground and put into a tea bag ready to use. The result of the determination of tannic acid content in beverages (80) showed that the content of tannic acid from brewed instant tea was higher than that from tea leaves. This information supported the use of tea leaves in

the present study. The use of tea leaves might be cause that made the inhibition was lower than the study by Morck *et al.*

Only one study demonstrated the effect of 300 ml of brewed tea. That was the study of Hurrell *et al* (55). Iron absorption reduced by 79 percent when subjects drank tea together with the meal. That was higher than this study, approximately 16-fold. The study of Hurrell *et al.* used black tea (3 g per 300ml), but the present study used 4.5 g of tea leaves to the same volume of water. Although the amount of tea in this study was more than that in the study of Hurrell *et al*, the inhibition by brewed tea in this study was lower. These different results might come from the effects of different types of tea because black tea was ground tea, so that brewed tea could release tannic acid more easily than tea leaves. Moreover, the test meal in the study of Hurrell *et al* was bread. It might have other chemical ingredients, such as enhancers and inhibitors, less than the test meal in the present study that was composed of rice and vegetables.

The results of this study and previous studies showed that the brewed tea from previous studies had greater effect than this study. However, it is not possible to compare the true different values because the previous studies examined the inhibitory effect of it *in vivo*, but an *in vitro* method was used in this study. Consideration of the unavailability of iron in the meal without brewed tea of both the present study (≈ 91% of total iron) and other previous studies (≈ 90% in study of Brune *et al*, 96% in the study of Morck *et al* and 96% in the study of Hurrell *et al*) found that were little differences. While the unavailability of iron in the present study was nearly the same, the meal without brewed tea in this study had higher tannic acid than other studies. Thus, it might possibly be that brewed tea had little effect on iron availability.

Another important factor was the content of tannic acid in the brewed tea added that would affect iron availability. The amount of tea, steeping time, kinds of tea i.e. black tea, green tea and herb tea, types of tea i.e. instant tea and tea leaves, source of the tea and quality of the tea, all of these might have effect on the content of tannic acid in brewed tea. Thus, the volume of brewed tea would not be appropriate to be used for discussion of the inhibitory effect of brewed tea, but we should consider the amount of tannic acid in the brewed tea and in the test meal together. The results of the present study showed that the greater volume of brewed tea added (200 and 300 ml), the less the inhibitory effect on iron availability in the test meals were found. One important aspect of the findings that could not be negligible was the ratio of tannic acid to iron in the meals. Because the inhibitory effect of brewed tea was possibly limited by the ratio of tannic acid to iron. It will be discussed in detail below (in 1.2)

1.2 The ratio of tannic acid and iron

The ratio of tannic acid and iron in the rice-based test meal after adding brewed tea was considered to explain the inhibitory effect of brewed tea on iron availability instead of the volume of brewed tea. The ratio of tannic acid and iron from the three types of rice based test meal, polished, mixed and brown rice-based test meals, were of quite similar values at each brewed tea volume added. The ratio of tannic acid and iron from the test meal without brewed tea added, 100, 200 and 300 ml were approximately 20:1, 30:1, 40:1 and 50:1, respectively. As the present study results, when the ratios of tannic acid and iron were 30:1, 40:1 and 50:1, iron availability from these test meals was lower than that from the test meal without brewed tea, but these inhibitions were not statistically significant.

The one previous study that could explain the present results was that of Brune *et al*, 1989 (7). Brune *et al* studied the relationship between the amount of tannic acid added to the meal and the degree of inhibition of iron absorption. Synthetic tannic acid was added in various amounts, 5, 10, 25, 50, 100 and 200 mg, to a bread meal which were given the ratio of tannic acid and iron be approximately 1:1, 3:1, 7:1, 13:1, 26:1 and 53:1, respectively. The smallest amount (5 mg on a ratio of 1:1) inhibited absorption by 20 percent and the rate of the decrease was more marked in the 5-50 mg range of tannic acid added (ratio of tannic acid and iron from 1.3:1 to 13:1) than at higher levels. When more than 50 mg of tannic acid was added, the inhibitions were no significant differences between the inhibition by 50, 100 and 200 mg of tannic acid or the ratio of tannic acid and iron as 13:1, 26:1 and 53:1, respectively. Results of the study of Seigenberg *et al* and Tuntawiroon *et al* supported the result of Brune *et al.* The study of Siegenberg *et al* found the reduction of iron absorption at ratios of tannic acid and iron higher than 11:1, 53:1 and 166:1, with no significant differences. Tuntawiroon *et al* examined the effect of a vegetable, Yod Kratin. There was a 50% decrease in iron absorption after the administration of as little as 3 g of Yod Kratin, corresponding to 87.6 mg tannic acid equivalent and iron absorption decreased more with each additional increase of Yod Kratin. However, that this further decrease was not statistically significant with more than 5 g additional Yod Kratin (10, 15 and 20 g), corresponding to 292, 438 and 584 mg tannic acid equivalent, or ratios of tannic acid and iron were 48:1, 79:1 and 101:1, respectively. The results from the previous studies showed the limit of inhibition by both synthetic tannic acid and food tannic acid on iron absorption that began to become saturated when the ratio of tannic acid and iron were high (higher than 13:1 in the study of

Brune *et al*; 11:1 in the study of Seigenberg *et al* and 28:1 in study of the Tuntawiroon *et al*). At these ratios, tannic acid could combine with iron until little iron remained. Thus, there were no significant differences between the inhibitory effect of tannic acid on iron absorption at higher ratios of tannic acid and iron.

The addition of brewed tea (100, 200 and 300 ml) into the test meal in this study made the ratio of tannic acid and iron was approximately 30:1, 40:1 and 50:1, respectively. Iron availability reductions when 100 ml of brewed tea (ratio of tannic acid and iron as 30:1) was added were 27, 18 and 17 percent in polished, mixed and brown rice-based test meals. When 200ml and 300 ml of brewed tea was added (ratio of tannic acid and iron as 40:1 and 50:1), iron availability reductions in the polished rice-based test meal were 14 and 4 percent, in the mixed rice-based test meal 10 and 4 percent, and in the brown rice-based test meal 9 and 3 percent. Comparing between the present study and the study of Brune *et al,* it was found that the ratios of tannic acid and iron of this study are high like those from the Brune *et al* study.

When the ratio of tannic acid and iron was considered, it was higher in the test meal without brewed tea in the present study (20:1) than the beginning ratio in test meals without added external tannic acid of the previous studies. At the beginning ratio of the present study, the iron content might almost combine with the tannic acid. So, when brewed tea was added, it did not significantly increase the inhibition of iron availability and there seemed to be little difference in these inhibitions. This result consistent with that in the previous studies at a higher level of tannic acid and iron ratio, 13:1 in the study of Brune *et al*, 53:1 in the study of Siegenberg *et al* and 48:1 in the study of Tuntawiroon *et al*. At ratios of tannic acid and iron higher than that, the

increase of inhibition was not significant; that might mean that the iron was combined with the tannic acid until it was almost saturated.

The other probable causes that made the inhibition by brewed tea in the present study lower than in the previous studies were the types of test meal and the source of tannic acid. Most of the previous studies, Brune *et al* and Seigenberg *et al*, used a bread meal that less contained other inhibitors or enhancers of iron absorption, such as phytate and vitamin C. Whereas the test meal in the present study composed of rice and vegetables that contained vitamin C which could enhance iron availability. Siegenberg *et al* (45) reported the addition of 50 mg or more of ascorbic acid to the test meal that contained 100 and 500 mg of tannic acid could increase iron absorption. The test meal in the present study had approximately 137 mg of vitamin C, and more tannic acid than the study of Siegenberg *et al*, about 3-fold. The higher vitamin C might decrease inhibitory effect of tannic acid on iron availability in diets. Apart from vitamin C, Brune *et al* and Siegenberg *et al* used synthetic tannic acid that caused strong inhibition (tannic acid, 5 mg, inhibited iron absorption by 20 percent) more than dietary tannic acid.

However, it is not possible to compare the true different values because the previous studies examined the inhibitory effect of tannic acid *in vivo*, but the present study used an *in vitro* method. In the same method, *in vivo*, there was a difference in iron absorption value among the previous studies because of the differences of the test meals and the iron status of the individual subjects.

There was a difference between the results of the present study and the three previous studies. In the present study, iron availability decreased at all levels of

brewed tea volume being added into the test meal. But adding a greater volume of brewed tea could inhibit iron availability less than adding a low volume of brewed tea. The study of Brune *et al*, Siegenberg *et al* and Tuntawiroon *et al* with high ratios of tannic acid and iron showed there was a still further decrease in iron absorption, that disagreed with the pattern of iron availability from the present study.

The pattern of results in the present study might be the result of iron availability that came from 2 parts. The first part was the iron from the test meal, of which there was the same amount in each test meal, and the second part was the iron from the brewed tea. The present study analyzed the iron content and iron availability from brewed tea. Iron from brewed tea had high availability (24.98 percent) which might be the cause of iron availability increasing when the volume of brewed tea was increased.

Gillies *et al*, 1983 (95) determined the mineral concentrations in brewed tea. They found brewed tea had copper, zinc, sodium and iron, and these were approximately 0.003, 0.01, 0.02 and 0.006 mg per 100 ml, respectively. In Thailand, Poonphonkul (63) reported brewed tea had copper 0.0037 mg, zinc 0.0144 mg and iron 0.0287 mg per 100 ml. Many previous studies investigated the effect of brewed tea in copper, zinc and sodium absorptions and they found the absorption of these was inhibited by brewed tea (96, 97). Greger *et al.* (96) studied the effect of tea in two forms, brewed tea and tea leaves, and found that these could inhibit the absorption of zinc. Thus, when the volume of brewed tea in the test meal was increased, the concentrations of other minerals increased too. These other minerals might be competitors to combine with tannic acid instead of iron so causing the iron to have

more chance of passing through the dialysis membrane and thus increasing iron availability.

Some previous studies reported the consumption of food high in tannic acid could cause thiamin deficiency. One cause of this deficiency is tea drinking and the chewing of fermented tea leaves (98, 99). Hilker *et al* (100) indicated tannic acid could react with thiamin to be a thiamin-tannic acid product and this product could not be absorbed. If we consider the amount of thiamin of the test meal in this study by using the information of the Thai Food Components Table List, we found there was little thiamin in the present test meal. However, the small content of thiamin might have some effect on tannic acid.

2. Iron availability from the different types of rice-based test meal with the same volume of brewed tea

With the same volume of brewed tea, all of the three types of rice-based test meal (polished, mixed and brown rice-based test meal) had the same pattern of iron availability. This pattern was that iron availability from the polished rice-based test meal was higher than from the mixed and brown rice-based test meal, and iron availability from the mixed rice-based test meal was higher than from the brown rice-based test meal. But the differences in iron availability for each type of rice-based test meal were not statistically significant ($p>0.05$).

However, the results of this study showed that the chemical compositions of the rice had an effect on iron availability. Two kinds of rice, polished and brown, had large differences in phytate content (approximately 2.5 fold). When the test meals were set up using different kinds of rice, the phytate content in each type of rice-based

test meal became different. The test meal with more phytate had lower iron availability than the test meal with less phytate. The present result agrees with previous studies (34, 35, 101-102). Siegenberg *et al* (45) studied the effect of phytate phosphorous (from 10 to 58 mg); iron absorption decreased more when the amount of phytate was increased (from 11.8 to 53 percent). The difference between the present study and the study of Siegenberg *et al* was the type of test meal. Siegenberg *et al* used synthetic phytate and a white-bread meal without enhancers or inhibitors. Thus, this previous study had a strong inhibitory effect from phytate, whereas this study used rice and vegetables, which had other chemical compositions apart from phytate. The other chemical compositions might interfere with the effect of phytate on iron availability, such as vitamin C.

Boonritthikarn (102) found that phytate had the effect of decreasing significantly the iron availability in food. When the two studies are compared (this study and the study of Boonritthikarn), they both had similar test meals that were composed of rice and vegetables, and they had similar contents of phytate, approximately 1,100–1,500 mg in the present study and 1,200–1,600 mg in the study of Boonritthikarn. The difference in the two studies was the content of vitamin C. This test meal had more vitamin C than the previous study, approximately 5-fold. This difference might be what made the different amount of phytate in each type of rice–based test meal have little inhibitory effect on iron availability. Vitamin C could enhance iron availability. Hallberg *et al* (35) reported vitamin C, or ascorbic acid, could strongly inhibit the effect of phytate on iron absorption.

The addition of brewed tea to the test meal caused it to have more other minerals that were contained in the brewed tea, such as copper, zinc and sodium (103-106). These other minerals could combine with phytate, especially zinc (106). Lönnerdal reported phytate in cereal, corn and rice inhibited zinc absorption strongly; that supported the result of Sandstorm *et al.* (104) and Sandstead *et al* (107). Regarding the effect of phytate on absorption of other minerals, it might be that phytate did not have a clear effect on iron availability, because one phytate in each type of rice-based test meal was combined with other minerals. The remaining phytate was not enough to make any significant difference to the inhibitory effect.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

The present study investigated the effect of brewed tea on iron availability from rice-based test meals. The rice-based test meals were set into three types depending on the kinds of rice; particularly polished, mixed and brown rice. Each type of test meal was composed of different rice and the same vegetables. For the polished rice-based meal, 60 g polished rice was included. Sixty gram of mixed rice (polished rice:brown rice 1:1) was for the mixed rice-based meal, and 60 g brown rice was for the brown rice-based meal. The same kinds and amounts of vegetables, 150 g string bean, 100 g kidney bean and 50 g Thai spinach were included in each meal, leading to having the same amount of substances related to iron absorption, crude fiber and total iron. All kinds of raw rice and vegetables were analyzed for chemical composition related to iron availability; total iron, crude fiber, vitamin C, tannic acid and phytate. The three cooked rice test meals with adding brewed tea were analyzed for available iron. The results of the present study follow below:

1. Among the chemical compositions of the rice-based test meals, total iron contents in polished, mixed and brown rice-based test meals were 4.06±0.5, 4.11±0.9 and 4.23±0.4 mg, and the crude fiber contents were 4.10±0.2, 4.25±0.2 and 4.30±0.2 mg, respectively. These contents were close to 1/3 of the recommended daily requirement which were 5 mg total iron and 5 g crude fiber. The total iron and crude

fiber contents between the three types of rice-based test meal were not statistically significant ($p>0.05$). Other chemical contents, vitamin C and tannic acid, were approximately 133.5 and 83.5 mg in each type of rice-based test meal. The differences in vitamin C and tannic acid content between the three types of rice-based test meal were not significant ($p>0.05$). The only chemical content which showed a significant difference ($p<0.001$) between polished, mixed and brown rice-based test meal, was phytate. The phytate content of polished rice-based test meal (1075.00± 19.8 mg) was substantially lower than that from mixed (1277.18±19.0 mg) and brown rice-based test meals (1479.03±23.7 mg). The difference was the result of the phytate contents of the rice used, because brown rice (1152.90±2.51 mg/100 g) had approximately 5 times more phytate than polished (480.38±1.06 mg/100 g).

2. The brewed tea in the present study had iron 0.1229±0.0 mg/100 ml, and iron availability was 24.98±0.6 percent. Double distilled water was used to brew the tea, thus the iron content in brewed tea was iron from the tea only. The different content of total iron and tannic acid content in brewed tea between the present study and other previous studies depended on types and kinds of tea, brewing method and water type used for brewing as well.

3. Before adding brewed tea into a test meal, the percentage of available iron to total iron in each type of rice-based test meal was different. The proportions from the polished rice-based test meal (9.8862±0.02 % of total iron) was higher than that from the mixed rice-based test meal (8.8170±0.02 % of total iron) and from the brown rice-based test meal (8.6544±0.02 % of total iron). These findings were because brown

rice had high phytate content, resulting in the lower iron availability in the test meal containing brown rice.

4. When brewed tea was added (100, 200 and 300 ml) the reductions of iron availability from the polished rice-based test meal were 27, 14 and 4 percent; from the mixed rice-based test meal they were 18, 10 and 4 percent; and from the brown rice-based test meal they were 17, 9 and 6 percent. However, the reductions of iron availability from all types of rice-based test meal at all volumes of brewed tea were not statistically significant ($p>0.05$). The probable cause of this finding was the high ratio of tannic acid and iron (20:1) in test meal at the beginning without adding brewed tea in this study. At this ratio, nearly all of iron in the test meal might bind to tannic acid. Thus, when brewed tea was added into the test meal, the tannic acid in brewed tea could not affect the iron availability because no more iron was bound to tannic acid..

The result of the present study was consistent with the previous study. Brune *et al* (7) found there were no significant differences between the inhibitory effects at ratios of tannic acid and iron in meals higher than 13:1. At this ratio, iron might combine with tannic acid until it is almost saturated. Thus, when brewed tea was added, it did not increase the inhibition of iron availability. However, the present result differed from Brune's study which found that the inhibitory effect decreased when more brewed tea was added. This was considerably caused by the high available iron in the brewed tea (24.98±0.6%), resulting in the less decrease of iron availability when volume of brewed tea was increased.

5. When the iron availability from the three types of rice-based test meal was considered, the iron availability from the polished rice-based test meal was higher than that from the mixed or brown rice-based test meals at the same volume of added brewed tea, but not significantly so ($p>0.05$). The test meal in the present study had high vitamin C, approximately 133.5 mg, which might be one cause of iron availability from each type of rice-based test meal showing no difference, because vitamin C could affect the inhibitory effect of phytate in the brown rice containing test meals.

Recommendations

1. From the present study, the high tannic acid : iron ratio was high in the test meal which was composed of rice and vegetables. Thai meals contain large components of rice and vegetables may be high in proportion of tannic acid to total iron which affect iron availability. Up to now, data of tannic acid content in Thai food is not available. For further research, the content of tannic acid in various Thai foods is needed to determine in order to estimate the amount of tannic acid in a meal consumed.

2. Although inhibitory effects of brewed tea on iron availability from rice-based test meals in the present study were not statistically significant, the available iron was shown to decrease with increasing brewed tea volume added. Thus, vulnerable people such as children, pregnant and lactating women, should not drink brewed tea after their meals. In addition, the kind of rice that was used in the test meal had an effect on iron availability. However, vulnerable and vegetarian groups who favor the

consumption of brown rice should consume a high amount of iron and vitamin C to reduce the chance of being iron deficient.

3. Apart from the high amount of tannic acid and vitamin C in the test meals, which might be major causes of making brewed tea have no significant effect on iron availability, iron availability might depend on other factors contained in the meal. Thus, further research should determine what the other factors are.

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APPENDIX A

Determination of crude fiber

1. Reagents

1.1 Sulfuric acid solution 1.25%

1.2 Sodium hydroxide solution 1.25%

1.3 Ethyl alcohol 95%

2. Procedure

2.1 Transfer 0.2 g of defatted dry sample to 500 ml flask. Add 200 ml of the 1.25% H_2SO_4 solution, immediately connect digestion flask with condenser, and heat to boiling for exactly 30 minute. Rotate flask frequently until sample is thoroughly wet. Take care to keep material from remaining on sides of flask out of contact with solution. Maintain constant volume of solution throughout digestion.

2.2 After 30 minutes, remove flask, immediately filter through linen in fluted funnel, wash with boiling water until no acid in seen.

2.3 Wash sample from linen with wash bottle back into flask with 200 ml 1.25% NaOH solution, using wash bottle marked to deliver 200 ml. Connect flask with reflux condenser and boil exactly 30 minutes. Maintain constant volume of solution throughout digestion.

2.4 After 30 minutes, remove flask, immediately filter through Whatman filter

paper No. 1 which know constant weigh. After throughly washing residue with boiling water, wash with 95% alcohol.

2.5 Dry filter paper and contents at 105°C for 2 hours. Cool in desiccator and weigh.

2.6 Ash 2 hours at 550±10°C, cool in desiccator, and weigh.

2.7 Calculate the content of crude fiber per fresh weight by

$$\%\text{crude fiber} = \frac{[\text{weight of residue} - \text{ash weight}] \times (100 - \%\text{moisture})}{\text{weight of sample}}$$

APPENDIX B

Determination of vitamin C

1. Reagents

1.1 2,4-dinitrophenylhydrazine reagent: dissolve 2 g of the reagent in 100 ml of 9 N sulfuric acid. Allow to stand overnight and filter.

1.2 Oxalic acid 0.5%

1.3 Trichloroacetic acid solution 4%

1.4 Acid-wash norite: place 200 g norite in a large flask. Add 1000 mlof 10 percent hydrochloric acid and heat to boiling. Filter with suction. Remove norite cake to large beaker and add 1 liter distilled water. Stir thoroughly and filter. Repeat until washings give a negative or very faint test for ferric iron (test filtrate with 1 percent potassium ferrocyanide). Dry norite cake in oven overnight at 110-120°C.

1.5 Sulfuric acid, 85 percent solution: to 100 ml to distilled water add 900 ml of concentrated sulfuric acid (specific gravity 1.84). Do this mixing very carefully in a sink.

1.6 Sulfuric acid 9 N: add cautiously 250 ml of concentrated sulfuric acid (specific gravity 1.84) to 700 ml of distilled water, cool and dilute to 1000 ml with distilled water.

1.7 Thiourea, 10 percent solution: dissolve 10 g of thiourea in 100 ml of 50 percent (by volume) aqueous ethyl alcohol. This reagent keeps satisfactorily for 2 months, but to check, see that it readily reduce $HgCl_2$ or $KMnO_4$.

1.8 Stock vitamin C standard (U.S.P. reference standard if available): dissolve exactly 100 mg L-ascorbic acid in 100 ml 4% trichloroacetic acid solution (1 mg per ml).

1.9 Working standard: dilute 2 ml of stock standard to 100 ml with 4% trichloroacetic acid, adding 1 ml of thiourea solution prior to diluting to volume (20 mg per ml).

2. Standard curve

To 25 ml of working standard L-ascorbic acid in 50 ml erlenmeyer flask add one-half teaspoon of norite. Shake for 1 minute and filter. Set up a standard curve using 0 to 3.0 ml of this filtrate at 0.5 ml intervals and dilute each to 4 ml with 4% trichloroacetic acid. Continue as under "development of color", below.

3. Procedure

3.1 Dilute 2 g of food in a 100 ml volumetric flask to volume with 0.5 percent oxalic acid.

3.2 Mix in a blender for the minimum time necessary.

3.3 To 20 ml of the homogenate in a 50 ml centrifuge tube add one-half teaspoon of norite, shake virgorously for 1 minute and filter through Whatman no.42 filter paper.

4. Development of color

4.1 Add exactly 4 ml of the filtrate to each of three boiling tubes.

4.2 Add 1 drop of 10% thiourea solution. Reserve one tube as a blank.

4.3 To two other tubes add 1 ml of 2,4-dinitrophenylhydrazine solution and mix.

4.4 Place the tubes in a boiling water bath for exactly 10 minutes.

4.5 At the end of this time, immediately place the tubes in a beaker containing crushed ice.

4.6 Now add slowly, drop by drop, during a 1-1.5 minutes period, 5 ml of 85% sulfuric acid to triple tubes with virgorous mixing. Keep on abundant supply of crushed ice in the beaker.

4.7 To the blank tube add 1 ml of 2,4-dinitrophenylhydrazine solution.

4.8 Remove the tubes from the ice bath and allow to stand at room temperature for 10 minutes.

4.9 Set the instrument 100% transmittance at 515 nm with a tube of distilled water, and read the unknown and blank.

Subtract the density of blank from that of the unknown and calculate, using the standard curve. It is advisable to include a working standard in the range of values expected each time the method is run.

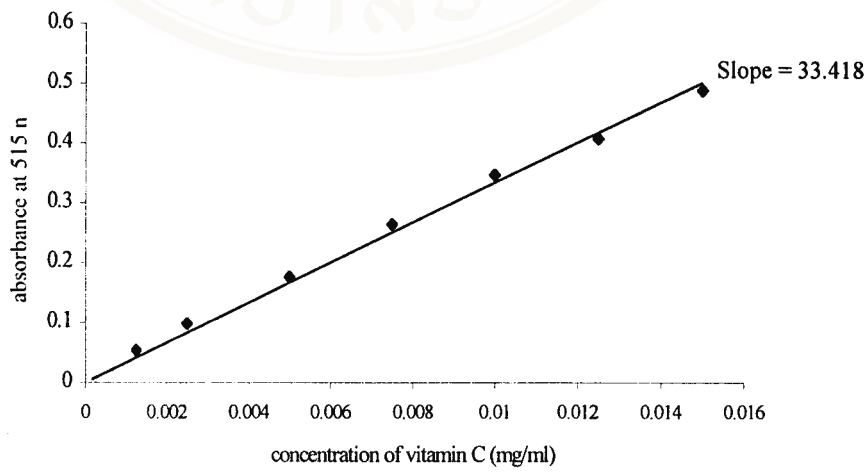

Figure 6 Standard curve of vitamin C

APPENDIX C

Determination of phytate

1. Reagents

1.1 Nitric acid 0.5 M

1.2 Ferric ammonium solution : dissolve ferric ammonium sulphate which containing 50 μg of iron in 1 ml solution.

1.3 Amyl alcohol

1.4 Ammonium thiocyanate : dissolve 100 g of ammonium thiocyanate in 1000 ml distilled water.

2. Procedure

2.1 One g of dried, finely-ground samples are extracted with 20 ml, 0.5 M HNO_3 for 3-4 hours with continuous shaking.

2.2 0.2-1.0 ml of the filtrate or standard sodium phytate solution is diluted with distilled water to a final 1.4 ml.

2.3 Add 1.0 ml of a solution of ferric ammonium sulphate containing 50 μ g. Fe is added to the diluted filtrate.

2.4 After mixing, the test-tubes are stopped and place in a boiling water-bath for 20 min.

2.5 Cool to room temperature, add 5 ml amyl alcohol to each test tube followed by 0.1 ml of a solution of ammonium thiocyanate.

2.6 The contents of the test tube are immediately mixed by inversion and shaking.

2.7 Centrifuging for a short time at low speed, the intensity of the color in the amyl alcohol layer is determined at 465 nm using a spectrophotometer against an amyl alcohol "blank", exactly 15 min after addition of the HN_4CNS.

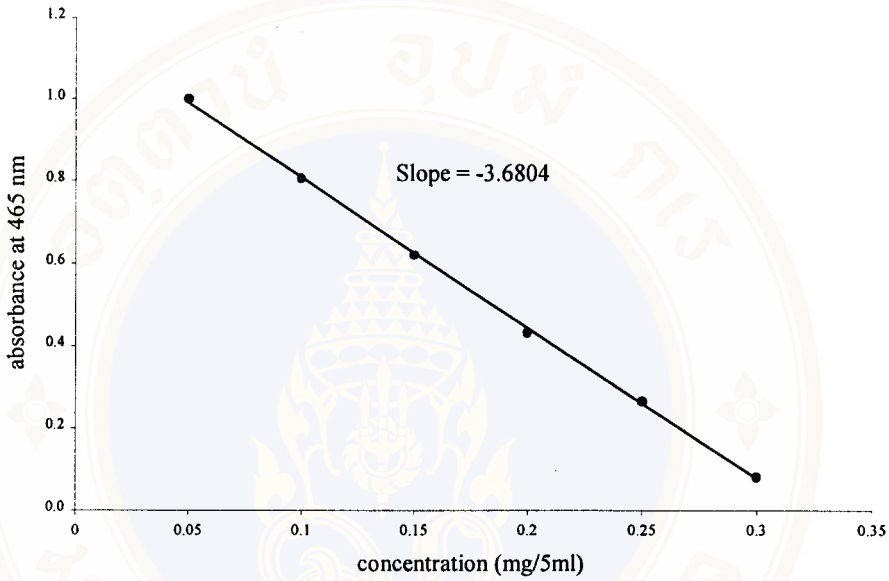

Figure 7 Standard curve of phytate

APPENDIX D

Determination of tannic acid equivalent

1. Reagents

1.1 50% dimethylformamide in 0.1 M acetate buffer, pH 4.4

1.2 FAS reagent: 89 parts of 50 per cent urea in an acetate buffer, 0.1 M, pH 4.4; 10 parts of 1 per cent arabic gum and 1 part of 5 per cent ferric ammonium sulphate in 1 M HCl

2. Procedure

2.1 Food samples (0.5-5 g) were extracted for 16 h with 50 ml of 50 per cent dimethylformamide in acetate buffer (0.1 M, pH 4.4) and then filtrated.

2.2 Two ml of the filtrate was mixed with 8 ml of FAS-reagent.

2.3 After 15 min, the absorbance were read at 578 and 680 nm against a reagent blank.

2.4 Food blanks were prepared by mixing 2 ml of the filtrate with 8 ml of FAS-reagent, made without iron. The food blank absorbances, at 578 and 680 nm, were subtracted from the experimental absorbances to give adjusted data.

2.5 Standard curves for catechin and tannic acid, 0.0-0.08 mg/ml, at both wavelengths, were used in calculating the content of catechol groups (expressed as catechin equivalents) and galloyl groups (expressed as tannic acid equivalents)(Figure 8-9)

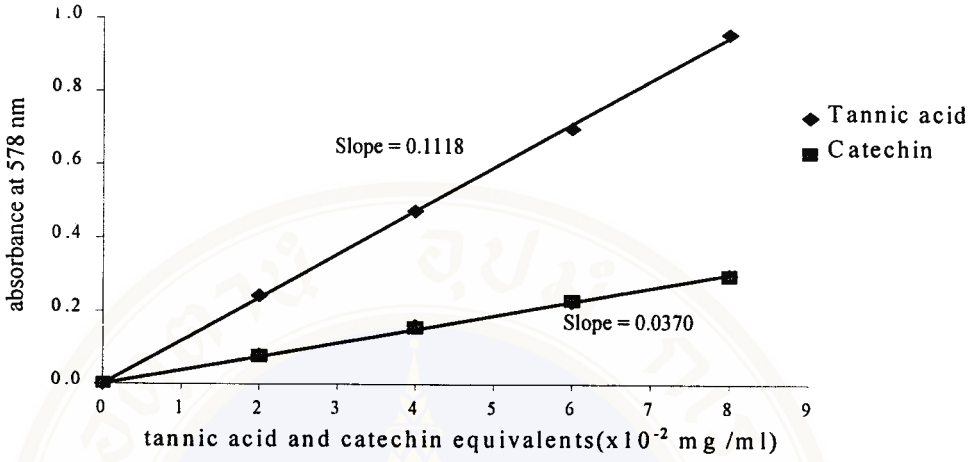

Figure 8 Standard curves of tannic acid and catechin at 578 nm

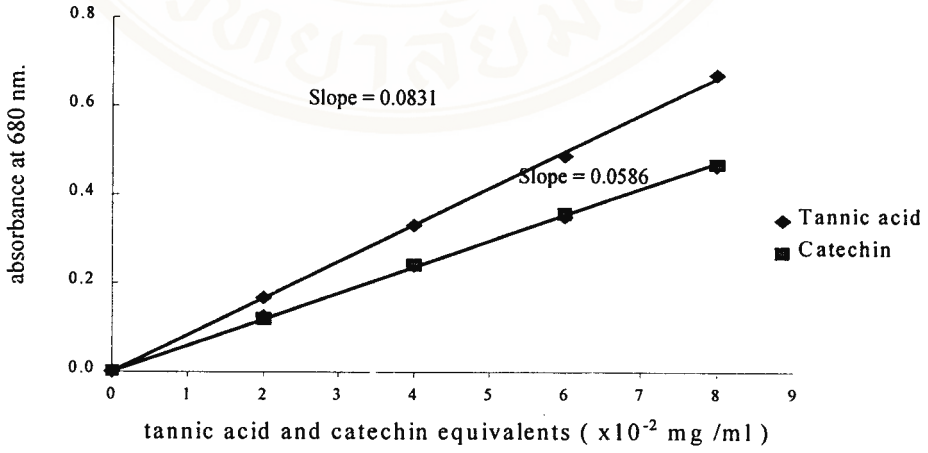

Figure 9 Standard curves of tannic acid and catechin at 680 nm

3. Realible method

3.1 Precision test

Guava pulp was used to determine the amount of tannic acid equivalent. The result of inter assay was shown in Table 18.

Table 18 The result of inter assay of tannic acid equivalent in guava pulp

Day	Tannic acid equivalent (mg/100g)	X	SD	% CV*
1	8.81			
2	9.84			
3	9.26			
4	8.53			
5	8.73	9.25	0.48	5.19
6	9.55			
7	9.83			
8	9.11			
9	9.05			
10	9.78			

*percent cv = (SD*100)/X

3.2 Accuracy test

The experiment which 1mg of tannic acid was added to guava juice was performed. The recovery was better than 80 percent is shown in Table.

Table 19 Recovery of tannic acid concentration from guava juice*

Guava juice plus tannic acid added (mg)	Actual recovery (mg/50ml)	% recovery**
Guava + 0	$0.469^{a}\pm0.005$	82.0
Guava + 1^{c}	$1.288^{b}\pm0.007$	

* Three aliquots per sample were analyzed and the mean value reported

** Percent recovery = [(b – a)/c] x100

APPENDIX E

Determination of total iron

1. Determination of total iron in test meal

1.1 Reagents

1.1.1 Concentrate sulfuric acid (H_2SO_4 conc.)

1.1.2 Hydrogen peroxide (H_2O_2)

1.2 Procedure

1.2.1 0.5-1 ml of concentrated H_2SO_4 and a few drop of hydrogen peroxide (100 vol. Analar) were added in 5 ml of aqueous homogenate, and heated gently until digestion was complete.

1.2.2 Twenty ml of iron-free water was added and after boiling the final volume was increased to 50 ml.

1.2.3 The iron concentration was measured by atomic absorption technique.

2. Determination of total iron in raw material

2.1 Reagents

2.1.1 HCl 6 N

2.1.2 HNO_3 0.1 M

2.2 Procedure

2.2.1 Sample 5 g was placed in crusible and heated until then becoming ash at 450°C.

2.2.2 Five ml of 6 N HCl was added in crusible, heated until HCl dried.

2.2.3 Added 25 ml of 0.1 M HNO_3 and stood at room temperature for 2 hours.

2.2.4 After 2 h, filtered with filter paper and analyzed total iron in the filtrate by atomic absorption technique.

APPENDIX F

Determination of available iron

1. Reagents

1.1 Pepsin solution: Ten g of pepsin (from hog stomach mucosa. Sigma Chemical Co, no P7000, St Louis, MO) was brought to 100 ml with 0.1 N HCl.

1.2 Pancreatin-bile suspension: Pancreatin, 0.8 g (porcine pancreas, Sigma Chemical Co, no P7000, St Louis, MO) and 5 g bile extract (Porcine, Sigma Chemical Co, no B8631, St Louis,MO) was suspended in 200 ml of 0.1 M $NaHCO_3$.

1.3 HCl 6 N and HCl 0.01 N

1.4 KOH 0.5 N

1.5 $NaHCO_3$ 0.5 N

1.6 Dialysis tubing molecular weight cut off 6,000-8,000

2. Procedure

2.1 Homogenized test meal until that homogenous.

2.2 Homogenous test meal was placed in a 500 ml Erlenmeyer flask.

2.3 HCl 6 N was added to the test meal to adjusted pH as 2.0±0.05 and the flask contents were brought to a total weight of 100 g with 0.01 N HCl.

2.4 Five ml of pepsin solution were added to the flask. The flask was inverted several time to mix and incubated for 2 h in a 37°C shaking water bath.

2.5 After the 2-h pepsin digestion, the content in flask was subdivided into two 20-g aliquots. One of these were frozen.

2.6 The second was mixed with 5 ml of the pancreatin-bile suspension and titrated to pH 7.5 with 0.5 N KOH

2.7 Dialysis sac containing a volume of 0.5 N $NaHCO_3$ equal to the volume of 0.5 N KOH required to titrate the aliquot-pancreatin-bile mixture to pH 7.5, plus water to bring the total volume to 20 ml, were prepared to product.

2.8 The frozen aliquot was thawed in a 37°C water bath anda dialysis sac containing the appropriate number of bicarbonate equivalent was added to each.

2.9 The aliquot was incubated in a 37°C shaking water bath until the product had pH 5

2.10 Five ml of pancreatin-bile suspension was added and incubation was continued for 2 hours. After the pancreatin digestion, aliquot from the dialysis sac (dialysate) was analyzed for available iron by atomic absorption technique.

BIOGRAPHY

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